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SOME EFFECTS OF CHILLING TEMPERATURES ON SWEETPOTATOES[1]

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INTRODUCTION

Sweetpotatoes (*Ipomoea batatas* (L.) Poir.) deteriorate rapidly after certain periods of exposure to temperatures ranging from −2° C., the freezing point of the roots,[2] to about 9°. The obvious deterioration is due to fungi, but whether or not they are the primary cause was not known when the present investigation was undertaken (1920). There was a theory extant to the effect that sweetpotatoes were injured, independently of infection by microorganisms, when exposed to chilling[3] temperatures; but no data were available to support this theory.

A number of fungi, the more important being *Mucor racemosus* Fres., *Botrytis cinerea* Pers., *Alternaria* sp., and *Penicillium* sp., cause decay of sweetpotatoes[4] in from three to six weeks when stored at temperatures from 0° to 8° C. The temperature ranges in which these organisms have been shown[4] to cause decay are as follows: *Mucor racemosus*, from 0.4° to 8°; *Botrytis cinerea*, from 2.4° to 20.9°, the highest temperature employed; *Alternaria* sp., from 7.9° to 14.4°; and *Penicillium* sp., from 4.4° to 12°. These temperature relations were obtained from experiments in which the roots were inoculated with pure cultures of the organisms, in the case of *Mucor racemosus*, *Botrytis cinerea*, and *Penicillium* sp., by the "well" method;[4] and in the case of *Alternaria* sp., by inserting mycelium and spores into the root ends.

If sweetpotatoes are exposed to various temperatures without inoculation, the normal range for infection for all four organisms is about −1° to 9° C. This normal temperature range for infection does not correspond with the temperature ranges for growth on culture media of any of the organisms. The temperature ranges for growth of *Penicillium* sp. and *Alternaria* sp. have not been studied, but both of these organisms grow readily at room temperatures (20° to 22°). The range of temperatures at which *Mucor racemosus* will grow on sweetpotato agar extends from approximately 0° to 33° (optimum about 25°), and that at which *Botrytis cinerea* will grow on carrot agar is from about 0° to 32° (optimum about 23° to 24°). There is also a

[1] Received for publication Nov. 1, 1930; issued May, 1931.

[2] WRIGHT, R. C., and TAYLOR, G. F. THE FREEZING TEMPERATURES OF SOME FRUITS, VEGETABLES, AND CUT FLOWERS. U. S. Dept. Agr. Bul. 1133, 8 p. 1923.

[3] As used in this paper, the term "chilling" applies to temperatures between the freezing point of sweetpotato roots (about −2° C.) and 9°. These temperatures are usually regarded as unfavorable for the storage of sweetpotatoes.

[4] HARTER, L. L., WEIMER, J. L., and ADAMS, J. M. R. SWEETPOTATO STORAGE ROTS. Jour. Agr. Research 15: 337-368, illus. 1918.

discrepancy between the temperature ranges for growth and for infection of inoculated roots in the case of *Mucor racemosus*, *Penicillium* sp., and *Alternaria* sp. In the case of *Botrytis cinerea* no discrepancy is apparent. Although the range of temperature employed with this organism in the inoculation experiments did not extend above 20.9°, infection occurred at all the temperatures employed.

The foregoing data, particularly those relating to *Mucor racemosus*, indicate either that some change occurs in the roots at temperatures between the freezing point and 9° C. which makes them more susceptible to decay or that the pathogenes possess an ability to produce decay in sweetpotatoes at these temperatures that they do not have at higher temperatures. If this change in pathology as the temperature falls to 9° and below is due to the host, it may be regarded as the result either of injury or of a normal physiological process.

The purposes of the present investigation were (1) to determine whether it is possible, by modifying the humidity of the storage chamber at temperatures below 10° C., to prevent infection by the organisms listed above, and (2) to see if sweetpotatoes are injured at these temperatures.

MATERIAL AND EQUIPMENT

In the experiments conducted prior to the season of 1926–27 the Yellow Jersey variety of sweetpotatoes was used; subsequently, the Yellow Jersey Improved.[5] The sweetpotatoes were grown on the Arlington Experiment Farm, near Rosslyn, Va. They were cured and stored in a sweetpotato storage house constructed according to United States Department of Agriculture plans.[6] The curing temperature varied in different lots used, but the extreme variations were between 23° and 30° C. The subsequent storage temperature varied from 10° to 15°.

The roots were exposed to the chilling temperatures, some in cold-storage rooms 8 feet wide, 14 feet long, and 11.5 feet high, and others in galvanized-iron chambers 35 inches wide, 42 inches long, and 40 inches high, which occupied three of the cold-storage rooms. At the termination of the chilling period the roots, not examined for injury at the time, were stored in a sweetpotato storage house or in infection chambers described elsewhere.[7]

CONTROL OF TEMPERATURE AND HUMIDITY

The temperatures in all the chilling rooms, except those in which the chambers were installed, were maintained by means of brine refrigeration and controlled by hand. In addition to thermograph records, thermometer readings were taken twice a day for each room. The humidity of the two rooms in which the sweetpotatoes were chilled during the seasons of 1927–28 and 1928–29 was governed in one room by calcium chloride in evaporation pans and in the other by means of a commercial humidifier. The relative humidities of these two rooms were determined from psychrometer readings taken twice daily and from hygrothermograph records.

[5] LAURITZEN, J. I. A STRAIN OF YELLOW JERSEY SWEETPOTATO RESISTANT TO SURFACE ROT (FUSARIUM OXYSPORUM W. AND C.). Jour. Agr. Research 33: 1091–1094. 1926.

[6] THOMPSON, H. C. SWEET POTATO STORAGE. U. S. Dept. Agr. Farmers' Bul. 970, 27 p., illus. 1918.

[7] LAURITZEN, J. I., and HARTER, L. L. SPECIES OF RHIZOPUS RESPONSIBLE FOR THE DECAY OF SWEETPOTATOES IN THE STORAGE HOUSE AND AT DIFFERENT TEMPERATURES IN INFECTION CHAMBERS. Jour. Agr. Research 24: 441–456, illus. 1923.

In the rooms where the galvanized-iron chambers were located the air was cooled by brine refrigeration and heated back to the desired temperatures by electric heating coils, and the temperature was controlled by mercury thermoregulators. The temperature of the chambers was the same as that of the room in which they were located. The humidity of the chambers was governed by means of calcium chloride and water in evaporation pans. Wet-bulb and dry-bulb readings were made through glass windows in the doors of the chambers from a psychrometer located just back of the windows. The necessary evaporation from the wet bulb was brought about by means of a fan blowing directly on the bulb.

EXPERIMENTAL DATA

The experimental work reported herein was carried on over a period of years extending from 1920 to 1929. The early work was limited because the desired conditions of humidity were not available. In the later experiments a variety of conditions of temperature and humidity became available and it was possible to isolate more definitely the effects of chilling temperatures.

A large number of trials made during the period extending from 1920 to 1922 and throughout the storage season showed that sweetpotatoes could be exposed to temperatures of 0°, 2°, and 4.5° C. for a period of 10 days and less without infection or apparent injury. Nor was infection or injury manifest after months of later storage in a sweetpotato house at temperatures ranging from 10° to 15°. In 1922 Gardner [8] reported experiments in which he exposed sweetpotatoes to temperatures as low as 0° for 12 hours without evident injury either immediately following chilling or after a period of storage at higher temperatures.

A large number of experiments conducted from 1922 to 1926 gave the same results consistently for the same temperatures and the same periods of exposure. Even when sweetpotatoes were stored at the freezing temperature of the roots (about −2° C.) long enough for some of them to freeze, the unfrozen roots, when subsequently stored for months at temperatures ranging from 10° to 15°, showed no apparent injurious effects.

Quantities of roots have been stored at temperatures ranging from 0° to 5° C. for 15 to 20 days in ordinary cold-storage rooms with very little infection or any evidence of injury that at the time could be dissociated from infection, either at the end of the chilling period or after a later period of storage at the usual storage temperatures. As the chilling period was extended beyond 20 days all the roots gradually became infected. It was not possible in the presence of such infection to determine whether or not there was injury. An effort was therefore made to see if it was possible to prevent infection by modifying the humidity of the storage chamber and thus permit of a study of possible injury.

During the storage season of 1926–27 the following nine combinations of temperatures and relative humidities were available: Relative humidities of 62, 80, and 96 per cent, each at temperatures of 9.5° [9] and 4.5° C., and relative humidities of 64, 79, and 95 per cent at 0.1°.

[8] GARDNER, W. A. SOME NOTES ON THE PHYSIOLOGY OF SWEETPOTATOES. Sweet Potato Bul. 2 (6): 7–10. 1922.

[9] The roots stored at 9.5 °C. may be regarded as checks on the roots stored at the two lower temperatures. It would have been preferable to use a slightly higher temperature, because 9.5° is encroaching on the range of temperatures (below 10°) regarded as inimical to successful storage of sweetpotatoes.

Sixteen lots of 10 roots each of the Yellow Jersey Improved were stored in 4-quart till baskets at each of the above combinations of temperature and humidity. Two baskets were removed from each of the nine conditions at the end of 5, 10, 15, 20, 29, 40, 60, and 70 days. After the roots were examined for data on disease, one of the baskets from each condition was stored in a sweetpotato storage house (temperature 10° to 25° C.) for various periods. The uninfected roots from the other baskets were examined for internal injuries, and isolations were made from infected roots.

After various periods the stored half of the chilled roots were examined for the occurrence and amount of decay in the infected roots and for internal injury and percentage of germination in the uninfected roots.

INFECTION DATA

The data from the 1926–27 experiment regarding infection are recorded in Table 1. No infection by fungi that normally cause decay of sweetpotatoes at temperatures below 9° C. occurred at any of the relative humidities at 9.5°, during either the chilling periods or the subsequent storage periods. However, some infection with surface rot, caused by *Fusarium oxysporum* Schlecht. did occur. Infection by the fungi that normally cause decay at the chilling temperatures occurred at temperatures of 0.1° and 4.5° at all the humidities by the end of the chilling periods of 60 and 70 days, and at some of the humidities by the end of 20, 29, and 40 days. The full effects of the exposure to these temperatures for periods of 15 to 70 days were not manifest in some cases at the end of the chilling period, but became evident by the end of the subsequent storage period. It is certain that macroscopically invisible infections were incipient at the end of some of these chilling periods or that some infection occurred during the storage period, because not only was the percentage of infection larger in certain cases following the storage period, but it then became evident in some roots that had been subjected to the shorter chilling periods, thus having the effect of shortening the chilling period required for infection to occur.

No infection occurred after chilling periods of 5, 10, and 15 days at 0.1° and 4.5° C., and only 5 per cent at 4.5° after 20 days' chilling. No infection occurred after the storage periods subsequent to chilling in roots chilled for 5 and 10 days, and very little occurred in roots chilled for 15 and 20 days. Although there was no infection in 29 days of chilling at 4.5° with a relative humidity of 62 per cent, or in 29 or 40 days of chilling at 0.1° with relative humidities of 64 and 79 per cent, a rather high percentage of infection occurred in each case after a subsequent period of storage. After chilling for 60 and 70 days at all the humidities at temperatures of 0.1° and 4.5° all the roots were infected at the end of the storage periods. However, at the end of the chilling periods the percentages of infection were less in a number of these combinations of temperature and humidity than at the end of the storage periods.

TABLE 1.—*Influence of chilling temperatures and relative humidities on infection of sweetpotato roots by certain fungi* [a] *(season of 1926–27)*

Chilling temperature	Relative humidity	Total roots	Roots infected after indicated chilling period of days stated							
			5	10	15	20	29	40	60	70
°C.	*Per cent*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
9.5	62	20	0	0	0	0	0	0	0	0
9.5	80	20	0	0	0	0	0	0	0	0
9.5	96	20	0	0	0	0	0	0	0	0
4.5	62	20	0	0	0	0	0	25	80	60
4.5	80	20	0	0	0	0	10	50	95	100
4.5	96	20	0	0	0	5	30	90	100	100
.1	64	20	0	0	0	0	0	0	40	95
.1	79	20	0	0	0	0	0	0	30	75
.1	95	20	0	0	0	0	5	75	100	100
			Roots infected after indicated chilling and storage [b] period of days stated							
			5 and 77	10 and 72	15 and 67	20 and 62	29 and 53	40 and 42	60 and 22	70 and 12
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
9.5	62	10	0	0	0	0	0	0	0	0
9.5	80	10	0	0	0	0	0	0	0	0
9.5	96	10	0	0	0	0	0	0	0	0
4.5	62	10	0	0	10	0	40	50	100	100
4.5	80	10	0	0	0	0	80	70	100	100
4.5	96	10	0	0	0	0	90	100	100	100
.1	64	10	0	0	10	10	50	90	100	100
.1	79	10	0	0	0	0	50	70	100	100
.1	95	10	0	0	0	10	100	100	100	100

[a] Such as *Mucor racemosus, Botrytis cinerea, Alternaria* sp., and *Penicillium* sp.
[b] After the various chilling periods the roots were stored for the periods indicated at a temperature of 10° to 25° C. and a relative humidity of 70 to 85 per cent. Second number indicates storage period.

In roots chilled at a temperature of 4.5° C. the time required for the initial infection, as well as for infection of all roots, increased with the lowering of the humidity. In roots stored after they had become chilled at this temperature, the time required for infection to reach 100 per cent increased with the lowering of the humidity.

During the early stages of infection there was a direct correlation between the relative humidity and the percentage of infection in roots chilled, and in roots stored after they were chilled, at a temperature of 4.5° C., except when the roots were chilled for 15 days and stored for 67 days. In roots chilled at 4.5° but not stored there was an indirect correlation between the relative humidity and the period of time required to bring about infection. Such a correlation was not manifest in roots chilled at 4.5° and then stored.

In roots chilled at a temperature of 0.1° C., and in roots stored after chilling at this temperature, a relative humidity of 79 per cent was more effective in checking infection than one of 64 per cent.

The decay-producing organisms isolated from infected roots as they came from the chilling temperatures of 0.1° and 4.5° C. were *Mucor racemosus, Botrytis cinerea, Penicillium* sp., and *Alternaria* sp. Forms of Fusarium, in addition to the foregoing pathogenes, were obtained from infected roots stored after they had been chilled at 0.1° and 4.5°. The invasion of Fusarium was apparently through the lesions produced by the chilling-temperature pathogenes.

It was not possible by means of the relative humidities available to prevent infection by such organisms as *Mucor racemosus, Botrytis*

cinerea, *Penicillium* sp., and *Alternaria* sp. at temperatures of 0.1° and 4.5° C. However, it was possible to delay infection at these temperatures sufficiently to permit a study of the effects on the roots of longer exposures, aside from infection.

INTERNAL INJURY DATA

Throughout the entire period during which this work was conducted, 1920 to 1929, small percentages of the roots exposed longer than 20 days to temperatures of 0° to 5° C. have shown scattered areas of discolored tissue. These discolored areas, mostly brown (pl. 1) but sometimes tinged with black, were generally associated with the vascular tissue, but usually existed as local patches about the outer vascular tissue, rarely affecting it.

The results of the 1926–27 experiment are recorded in Table 2. Some symptoms of injury were found at all three temperatures, although only one root at 9.5° C. showed discoloration that may be attributed to the effect of this temperature, which is, of course, one-half degree lower than the minimum temperature (10°) recommended for the storage of sweetpotatoes.

TABLE 2.—*Chilling temperatures in relation to internal injury of sweetpotato roots (season of 1926–27)*[a]

Chilling period	Subsequent storage period	Roots chilled at—								
		9.5° C.			4.5° C.			0.1° C.		
		Total	Showing injury		Total	Showing injury		Total	Showing injury	
Days	*Days*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
5	0	30	0	0	30	0	0	30	0	0
10	0	30	0	0	30	0	0	30	0	0
15	0	30	0	0	30	0	0	30	0	0
20	0	30	0	0	30	0	0	30	0	0
29	0	[b]29	0	0	29	2	6.9	26	1	3.8
40	0	30	0	0	23	2	8.7	14	0	0
60	0	29	0	0	3	3	100	14	0	0
70	0	30	0	0	6	1	16.7	3	1	33.3
5	[c]77	30	0	0	30	0	0	30	0	0
10	72	30	0	0	30	0	0	30	1	3.3
15	67	30	0	0	29	0	0	29	1	3.4
20	62	30	0	0	30	0	0	28	2	7.1
29	53	29	1	3.4	9	0	0	10	4	40
40	42	13	0	0	8	1	12.5	4	2	50
60	22	8	0	0						
70	12	6	0	0						

[a] The data in this table were derived from the uninfected roots of the experiment from which the data in Table 1 were obtained.

[b] Thirty roots (10 roots from each of the 3 relative humidities) were used for each condition of time and chilling temperature, but in the final record only uninfected roots were counted in the internal injury data.

[c] At the end of the various chilling periods, the roots were stored for the periods indicated at a temperature varying from 10° to 25° C. and a relative humidity varying from 70 to 85 per cent.

The data obtained at temperatures of 0.1° and 4.5° C. are difficult to interpret because of the rare occurrence of the symptoms of injury in the form of discoloration and because of the erratic distribution of discoloration in relation to the treatment the roots received. Although there is a general tendency for the percentage of roots showing discoloration to increase with the increase in length of the chilling period, it is not marked, and there is one exception (compare the data obtained after 60 to 70 days of chilling at 4.5°). It is difficult to understand why the percentage of roots showing injury

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Sweetpotato roots exposed to a temperature of 0.2° C. for 45 days, showing brown areas
(Painting by J. Marion Shull)

should be larger in most instances at 4.5° than at 0.1° by the end of the chilling periods, when after a subsequent period of storage at 10° to 25° it was larger at 0.1° than at 4.5°. Since the total number of roots that showed symptoms of injury was small, it is possible that the distribution of the injury in the results obtained may have been influenced by the roots that were removed because of decay.

The shortest chilling exposure that resulted in symptoms of injury was 10 days at 0.1° C. This was in roots that were stored for 72 days after chilling. Otherwise signs of injury were not evident in roots chilled for only 10 days. Injury by chilling alone occurred after 29 days at both 0.1° and 4.5°.

The foregoing results were supplemented by two other experiments conducted on a limited scale during the storage seasons of 1927–28 and 1928–29. The results of these experiments are recorded in Table 3.

TABLE 3.—*Chilling temperatures in relation to internal injury of sweetpotato roots (seasons of 1927–28 and 1928–29)*

SEASON OF 1927–28

Chilling temperature	Relative humidity	Chilling period	Subsequent storage period [a]	Total roots	Roots showing injury
° C.	*Per cent*	*Days*	*Days*	*Number*	*Number*
9.5	83	21	0	10	0
4.5	72	21	0	10	0
.7	64	21	0	10	0
.2	50	21	0	10	0
9.5	83	45	0	10	0
4.5	72	45	0	10	0
.7	64	45	0	22	0
.2	50	45	0	20	3
9.5	83	21	79	25	0
4.5	72	21	79	23	0
.7	64	21	79	25	0
.2	50	21	79	8	1
9.5	83	45	54	23	0
4.5	72	45	54	5	0
.7	64	45	54	10	0
.2	50	45	54	0	0

SEASON OF 1928–29

10	89	20	0	10	0
4.5	75	20	0	10	0
1.2	69	20	0	10	0
0	67	20	0	10	0
10	89	40	0	[b] 0	0
4.5	75	40	0	0	0
1.2	69	40	0	0	0
0	67	40	0	20	1
10	89	20	37	20	0
4.5	75	20	37	17	0
1.2	69	20	37	20	0
0	67	20	37	36	1
10	89	40	36	15	0
4.5	75	40	36	13	0
1.2	69	40	36	5	1
0	67	40	36	0	0

[a] The storage temperature and relative humidity were 21° C. and 90 per cent, respectively.
[b] The uninfected roots held at temperatures of 10°, 4.5°, and 1.2° C. for 40 days were not examined for injury until after 36 days of storage.

In the 1927–28 experiment, quantities of Yellow Jersey Improved roots were exposed for 21 days and 45 days to temperatures of 9.5°, 4.5°, 0.7°, and 0.2° C. and to relative humidities of 83, 72, 64, and 50

per cent, respectively. The subsequent storage temperature was 21° and the relative humidity 90 per cent. No injury was observed after 21 days of chilling. After 79 days of subsequent storage one root showed injury among those chilled for 21 days at 0.2°. Three roots showed injury after being chilled for 45 days at 0.2°. The roots chilled at 0.2° for 45 days were all decayed after 54 days of storage. No injury was evident in any of the roots chilled for 21 or 45 days at the other temperatures.

In the experiment conducted during 1928–29, roots of the Yellow Jersey Improved variety were chilled for 20 and 40 days at temperatures of 10°, 4.5°, 1.2°, and 0° C. and at relative humidities of 89, 75, 69, and 67 per cent, respectively. The subsequent storage temperature was 21° and the relative humidity 90 per cent. No injury was evident by the end of 20 days of chilling at any of the temperatures. After 37 days of subsequent storage, one root showed injury among those chilled for 20 days at 0°. One root was injured after 40 days of chilling at 0°. The roots chilled for 40 days at 0° and stored for 36 days were all decayed by the end of the storage period. One root showed injury among those chilled for 40 days at 1.2° and stored for 36 days. No injury was found at temperatures of 4.5° and 10° after any of the chilling or storage periods.

The association of a brown or sometimes slightly black discoloration with the exposure of sweetpotato roots to temperatures from 0° to 4.5° C. is not always constant, or consistent with the length of the exposure, but it is believed that it is to be regarded as a sign of injury due to chilling. (See pl. 1.)

GERMINATION DATA

In the germination tests the chilled roots were stored under conditions favorable for sprouting. In the 1926–27 experiment the roots were stored in a sweetpotato storage house during the latter part of the storage season (from April 6 to June 22, 1927), when the temperature ranged from 10° to 25° C. and the relative humidity from 70 to 85 per cent. That these conditions were favorable for sprouting is shown not only by the sprouting of the general stock, but by the sprouting of the roots stored after being exposed to 9.5° during the various periods of chilling. (Table 4.)

The number of roots sprouting at 9.5° C. varied from 90 to 100 per cent. The 90 per cent count was obtained after 70 days of exposure, but since there was no further relation of the percentage of germination to the length of the period of exposure it is believed that this slight drop in the percentage of germination in the longest period of exposure has no significance. There is a difference of only 3 per cent between the percentage of germination after 70 days and that after 15 days of exposure, one of the time intervals between these, however, yielding 100 per cent germination.

The percentage of germination varied from 93 to 100 in roots chilled at 4.5° C. for 5, 10, 15, 20, and 29 days, and dropped to 75 in roots chilled for 40 days. In this last case the percentage involved the absence of germination of only 2 roots out of 8, and it is not certain, therefore, that this drop has any significance.

In the roots chilled at 0.1° C., the number that germinated dropped from 90 per cent of those chilled for 15 days to 57 per cent of those chilled for 20 days, and to 0 per cent of those chilled for 29 and 40

days. These results seem to prove that roots exposed to a temperature of 0.1° for periods longer than 15 days are injured independently of infection, and to suggest a possible injury to roots exposed to 4.5° for 40 days.

TABLE 4.—*Influence of chilling temperatures on the germination of sweetpotato roots (season of 1926–27)* [a]

Chilling period	Subsequent storage period [b]	Roots chilled at—								
		9.5° C.			4.5° C.			0.1° C.		
		Total	Germinating		Total	Germinating		Total	Germinating	
Days	*Days*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Number*	*Number*	*Per cent*
5	77	30	30	100	30	29	97	30	26	87
10	72	30	30	100	30	28	93	30	29	97
15	67	30	28	93	30	29	97	29	26	90
20	62	30	29	97	30	30	100	28	16	57
29	53	29	29	100	[c] 11	11	100	10	0	0
40	42	[d] 21	20	95	8	6	75	4	0	0
60	22	30	29	97						
70	12	20	18	90						

[a] The data in this table were derived from the uninfected roots remaining at the end of the experiment from which the chilling-period data in Table 1 were obtained.

[b] After the various chilling periods, the roots were stored for the periods indicated at a temperature of 10° to 25° C. and a relative humidity of 70 to 85 per cent.

[c] At temperatures of 4.5° and 0.1° C. each test was started with 30 roots (10 roots from each relative humidity), but in the final record only uninfected roots were counted in the germination data.

[d] Whenever the number of roots at 9.5° C. used in the counts was less than 30, the missing roots had been used previously for the purpose of making isolations from surface rot.

Germination data were obtained also in connection with the internal-injury data recorded in Table 3. These data are recorded in Table 5. After chilling, the roots in both experiments were stored at a temperature of 21° C. and a relative humidity of 90 per cent. The results in the main confirm those of the 1926–27 experiment. All the roots chilled at temperatures of 9.5° and 10° and those chilled at a temperature of 4.5° for 21 and 20 days in the 1927–28 and 1928–29 experiments, respectively, germinated. There was a drop from 100 per cent germination in roots chilled for 40 days at 10° (1928–29 experiment) to 77 per cent in those chilled at 4.5° for the same period. In the 1927–28 experiment, however, all the roots chilled at 4.5° for 45 days germinated. Thus, out of the three experiments (1926–27, 1927–28, and 1928–29), two (1926–27, Table 4, and 1928–29) showed a drop in the percentage of germination in roots chilled for 40 days at a temperature of 4.5°, while in the third (1927–28) there was no drop in germination in roots chilled for 45 days. In consequence of these inconclusive results, the question of injury in roots chilled at 4.5° remains uncertain.

The results given in Table 5 are consistent with those of the 1926–27 experiment (Table 4) in showing a drop in the percentage of germination below that obtained at 9.5° and 10° C. in roots chilled for 20 days and more at temperatures below 4.5°. The drop in the percentage germination in roots chilled for 20 days in the 1928–29 experiment was smaller than in those chilled for 21 days in the 1927–28 experiment. The difference in time of one day is not believed to be a factor. The decrease in the percentage of germination in the 1928–29 experiment would not in itself be convincing, but since it is consistent with all the other results, it is corroborative evidence of injury.

TABLE 5.—*Influence of chilling temperatures on the germination of sweetpotato roots, seasons of 1927–28 and 1928–29*

SEASON OF 1927–28

Chilling temperature	Relative humidity	Chilling period	Subsequent storage period [a]	Total roots [b]	Roots germinating	
°C.	*Per cent*	*Days*	*Days*	*Number*	*Number*	*Per cent*
9.5	83	21	79	25	25	100
4.5	72	21	79	23	23	100
.7	64	21	79	10	5	50
.2	50	21	79	8	0	0
9.5	83	45	54	23	23	100
4.5	72	45	54	5	5	100
.7	64	45	54	0	0	0
.2	50	45	54	0	0	0

SEASON OF 1928–29

10	89	20	37	20	20	100
4.5	75	20	37	20	20	100
1.2	69	20	37	19	18	95
0	67	20	37	45	35	78
10	89	40	36	15	15	100
4.5	75	40	36	13	10	77
1.2	69	40	36	5	4	80
0	67	40	36	0	0	0

[a] The storage temperature and relative humidity were 21° C. and 90 per cent, respectively.
[b] The roots used comprise the uninfected roots remaining at the end of the experiments from which the data in Table 3 were derived.

SUMMARY AND CONCLUSIONS

Sweetpotatoes of the Yellow Jersey Improved variety, when exposed for more than 20 days to temperatures ranging from 0° to 5° C. and relative humidities of 62 to 96 per cent, gradually became infected by such fungi as *Mucor racemosus*, *Botrytis cinerea*, *Penicillium* sp., and *Alternaria* sp. At temperatures from 0.1° to 4.5°, the percentage of infection was smaller (provided it had not reached 100 per cent) at the end of the chilling periods during which infection had occurred than after an additional period of storage at temperatures ranging from 10° to 25° C. Infection became evident during storage at a range of temperatures from 10° to 25° in certain cases where it was not evident at the end of the chilling periods.

In addition to the development of new lesions during the storage period, the old lesions became larger. These lesions apparently did not differ in appearance, aside from size, from those that developed during chilling. In addition to the above-mentioned fungi, forms of Fusarium were isolated from infected roots that had been stored at 10° to 25° C. after chilling at 0.1° to 4.5°. The invasion of Fusarium was apparently through the lesions caused by the other fungi.

During the chilling periods in which infection occurred, the percentage of infection in roots chilled at 4.5° C. was in general directly proportional to the relative humidity, as long as 100 per cent infection was not reached. At a temperature of 0.1° the percentage of infection was higher during its early stages at a relative humidity of 95 per cent than at the two lower humidities, both in roots only chilled and in roots stored for various periods after chilling. At a relative humidity of 79 per cent, however, there was less infection than at a relative humidity of 64 per cent.

The fact that sweetpotatoes are susceptible, at temperatures below 9° C., to infection which does not normally occur at temperatures above 9° may indicate a change in the physiology of the host or pathogenes, or it may mean that the host has suffered some injury. It seems probable that the change is in the host, since a simultaneous and parallel change in a number of pathogenes is hardly to be expected.

By lowering the relative humidity at temperatures of 0.1 to 4.5° C. from about 95 per cent to 80 and 62 per cent, it was possible to delay infection sufficiently to permit of an investigation designed to determine the presence and nature of any injury other than that caused by infection.

With the exception of one root which at 9.5° C. showed slight internal browning, suggestive of injury due to chilling, evidence of injury was not observed in roots held at temperatures ranging from 9.5° to 10°. Judging by internal discoloration, slight injury may occur in as little as 10 days' time in roots held at 0.1° and in 29 days in roots held at 4.5°. Only in the case of one root was injury observed in 10 days' time at 0.1°, after a subsequent storage period of 72 days. There is a tendency for the percentage of roots showing internal symptoms of chilling injury to increase with the length of the chilling period.

Evidence of injury was tested also by the ability of the chilled uninfected roots to germinate. In two experiments there was a drop in the percentage of germination in roots chilled for 40 days at 4.5° C., but in a third experiment no drop occurred after 45 days of chilling. Considering the small number of roots involved and the small drop in germination in the two experiments, together with the 100 per cent germination in the third, the evidence, while pointing to impairment of germination in roots chilled at 4.5°, remains, nevertheless, inconclusive.

In uninfected roots chilled at a temperature of 0.1° C. (1926–27 experiment) there was a decline in the percentage of germination, starting at the chilling period of 15 days, until there was no germination in roots chilled for 29 days. These results indicate that sweetpotato roots are injured when exposed to this temperature for 15 days or longer. The results of the germination experiments of 1927–28 and 1928–29 correspond closely with those of the 1926–27 experiment.

As a result of these findings, it would seem that the exposure of sweetpotatoes for periods less than 10 days to temperatures above the freezing point of the roots (−2° C.) and below 9.5° C. would not be injurious. A prolonged exposure, however, would subject the roots not only to the danger of infection by the various low-temperature fungi but also to chilling injury that may or may not be evident at the end of the chilling period. Such signs of injury may become evident after a period of storage even in a temperature favorable to sweetpotatoes.

Taking into consideration the danger of both chilling injury and infection, there is nothing to commend even the temporary exposure of sweetpotatoes to temperatures below 10° C.

EFFECT OF ABNORMALLY LONG AND SHORT ALTERNATIONS OF LIGHT AND DARKNESS ON GROWTH AND DEVELOPMENT OF PLANTS[1]

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INTRODUCTION

One of the striking facts developed in the study of plant response to relative length of day and night is that, so far as concerns initiation of flowering and fruiting, darkening the plant for a period in the middle of the day fails to produce the same effects as those resulting from excluding the early morning or late afternoon light. Early in the investigations of the present writers on this subject [2] it was shown that in such typical short-day plants as Biloxi soybeans (*Soja max* (L.) Piper) and *Aster linariifolius* L., darkening from 10 a. m. to 2 p. m. each day has but little effect in hastening flowering as compared with that produced by exposure to an unbroken short day. Further studies have shown that other short-day plants, as well as typical long-day plants, also show a curious indifference to darkening in the middle of the day with respect to flowering and fruiting, even when the amount of light thus excluded is vastly greater than that excluded with decisive effects in the early morning and late afternoon. These results indicate that, with a given total number of hours of daily illumination, two short periods do not produce the same result as a single unbroken period of illumination. Indeed, the effect of mid-day darkening is much the same as if the plants remained in the light for the whole day.

Another prominent feature of photoperiodic response in plants is that species and varieties differ widely in their sensitivity to change in length of day. Comparatively small changes in day length may exert marked formative action on some plants, while in other plants wide variation in the daily light period may produce only slight quantitative effects. The question naturally arises as to the character and extent of the effects that would be produced on the two classes of plants by alternations of light and darkness falling outside the range commonly experienced in nature. To obtain further information on this problem, with the results previously obtained by darkening soybeans and Aster in the middle of the day as a basis, the experiments discussed in this paper were undertaken. These experiments have been carried out on a number of plants of long-day, short-day, and indifferent types, with sunlight and with the tungsten-filament lamp as sources of illumination. A preliminary account of some of the earlier, less comprehensive tests appeared in 1927.[3]

[1] Received for publication Jan. 2, 1931; issued May, 1931.

[2] GARNER, W. W., and ALLARD, H. A. EFFECT OF THE RELATIVE LENGTH OF DAY AND NIGHT AND OTHER FACTORS OF THE ENVIRONMENT ON GROWTH AND REPRODUCTION IN PLANTS. Jour. Agr. Research 18: 553-606, illus. 1920.

[3] GARNER, W. W., and ALLARD, H. A. EFFECT OF SHORT ALTERNATING PERIODS OF LIGHT AND DARKNESS ON PLANT GROWTH. Science (n. s.) 66: 40-42. 1927.

METHODS OF PROCEDURE

Efforts were first directed toward amplifying the original results obtained with soybeans and Aster by testing other plants and by employing several modifications of the light treatment. It was desired to obtain as many data as possible with sunlight, bearing on the effects of alternations of light and darkness not commonly experienced by plants in nature, in order to furnish a check on the results obtained with artificial light. However, the opportunities for the use of sunlight in securing these alternations are rather limited because of the inevitable sequence of day and night in a cycle of 24 hours. In one series of tests the plants were subjected alternately to a 10-hour day and to the full day length of summer. For this and the two following series of tests with sunlight, the usual arrangement of light-proof houses and trucks on steel tracks for transferring the plants was employed. The midsummer day length at Washington, D. C., from sunrise to sunset, is slightly less than 15 hours.

In another series of tests conducted in midsummer the plants were darkened throughout the daylight period and exposed to full-day illumination on alternate days. In this way the alternations of light and darkness involved a cycle of 48 hours instead of the usual 24 hours. The plants were illuminated for about 15 hours in each 48-hour period, which would correspond to an average of 7½ hours of light daily. Along with these tests, another treatment involved daily darkening from 10 a. m. to 3 p. m. In this case the plants were illuminated for an average of about 9½ hours in each 24-hour period. In two instances the darkening was from 10 a. m. to 2 p. m. daily.

In the final series of tests with sunlight the plants were placed in the light-proof houses from 10 a. m. to noon and from 2 to 4 p. m. Thus they were exposed to three short periods of illumination each day, and the total daily illumination ranged from 11 hours to about 9 hours or slightly less, depending on the duration of the test.

To obtain systematic alternations of light and darkness of fixed duration it is necessary, of course, to employ artificial light. For the tests with artificial light a series of small light-proof compartments was used, each compartment being provided with a 1,000-watt Mazda lamp, with reflector, as a light source. The plants were protected from excess of radiant heat from the lamps by interposing a 2-inch screen of distilled water which was constantly recirculated from a tank by means of an electrically driven pump. A current of air was driven through the plant chambers at a uniform rate by means of a fan. The lights were turned on and off automatically at definite intervals by passing the electric current through a series of revolving drums having a segment cut away and fitted with copper brushes so as to provide for making and breaking the electric circuit. The time relationship between complete revolutions of the different drums was rigidly maintained by means of a series of gears, the whole being driven by a synchronous electric motor.

Except in the final tests, means were not at hand for maintaining constant temperature and humidity. Each series of tests, however, was essentially complete in itself, and thermographic records indicate that in each series the difference in the temperature of the different compartments was not at any time more than 1 or 2 degrees Fahrenheit. A fairly large number of experiments were carried out at

various temperatures and on various plant species. As an additional check, in some instances the alternations of light and darkness allotted to the different compartments were interchanged, in order to eliminate the effects of abnormal conditions that might exist in any compartment. In each series the mean temperatures were computed from the thermographic records. In the shorter alternations of light and darkness no changes in temperature due to the light coming on or going off could be detected. In the alternations of one hour or longer a rise of 1 or 2 degrees in temperature usually resulted when the lights came on. In the final tests a system of automatic control of temperature and humidity was in operation and, except on abnormally warm days, the temperature was held constant within $\pm 2°$ F., while the relative humidity was held constant within about ± 3 per cent.

By means of the water screen the proportion of infrared in the radiation from the lamp was reduced to a level considerably below that in sunlight. The visible portion of the spectrum of the tungsten filament lamp is deficient in the shorter wave lengths, as compared with sunlight. As regards intensity, values of 2,000 to 4,000 foot-candles were obtained immediately below the water screen, except at the outer edges near the walls of the compartment. The intensity decreased, of course, with increasing distance below the screen. The plants were grown in ordinary potting soil in wooden boxes. The containers were so adjusted as to bring the tops of the plants reasonably near the bottom of the water screen. Observations on the test plants were planned to show the effect of the different light treatments (1) on time of flowering and (2) on the nutrition and growth of the plants, as measured by appearance, height attained, and fresh and dry weights at the end of the test.

EXPERIMENTAL DATA

EXPERIMENTS WITH SUNLIGHT

In the first series of experiments, early, medium, and late varieties of soybeans and *Cosmos sulphureus* L., *Perilla frutescens* (L.) Britton, and *Impatiens balsamina* L. (garden balsam) were exposed alternately to a 10-hour day and to the full length of day during the latter part of June, July, and August. The control plants were exposed daily to the full length of day. Mandarin, the early variety of soybeans used, is relatively indifferent to seasonal changes in day length in the latitude of Washington. The three other varieties of soybeans and Cosmos and Perilla are to be classed as short-day plants, while garden balsam is a long-day type. Table 1 shows the results of the experiment with respect to date of first flowering and height attained by the plants at flowering.

In the next experiment, representative short-day and long-day plants were darkened throughout the daylight period and exposed to full-day illumination on alternate days. The controls were exposed to the full illumination period each day. Additional treatments included daily darkening of the plants from 10 a. m. to 3 p. m. or 10 a. m. to 2 p. m. and exposure to day lengths of 5, 8, 10, and 12 hours. The short-day plants used were Biloxi soybeans, *Tithonia tubaeformis* (Mill.) Blake, *Helianthus angustifolius* L., *Cosmos bipinnatus* Cav., and *Perilla frutescens* (L.) Britton. The long-day group

was represented by *Monarda didyma* L. and *Steironema ciliatum* (L.) Raf. The effects of the several treatments on date of first flowering and on average height of the plants are shown in Table 2.

TABLE 1.—*Time of flowering and growth of plants of the short-day, indifferent, and long-day types exposed on alternate days during the summer months to full-day and to 10-hour day illumination as compared with daily full-day illumination*

Type and species or variety	Treatment	First day of test	First flowering	Average height at flowering
				Inches
Short-day type:				
Peking soybeans	Full-day and 10-hour day alternations	June 29	July 24	6
Control	Full day daily	do	Aug. 10	12
Toyko soybeans	Full-day and 10-hour day alternations	do	Aug. 2	13
Control	Full day daily	do	Aug. 17	21
Biloxi soybeans	Full-day and 10-hour day alternations	do	Aug. 10	15
Control	Full day daily	do	Sept. 9	28
Cosmos sulphureus	Full-day and 10-hour day alternations	June 23	Aug. 30	38
Control	Full day daily	do	Oct. 15	
Perilla frutescens	Full-day and 10-hour day alternations	June 17	Aug. 11	24
Control	Full day daily	do	Sept. 9	30
Indifferent type:				
Mandarin soybeans	Full-day and 10-hour day alternations	June 29	July 23	7
Control	Full day daily	do	July 28	9
Long-day type:				
Impatiens balsamina	Full-day and 10-hour day alternations	June 17	July 12	9
Control	Full day daily	do	July 10	7

TABLE 2.—*Effects of complete darkening on alternate days and of daily midday darkening on time of flowering and growth of short-day and long-day plants, as compared with effects of exposure to the full day and to various short days*

Type and species or variety	Duration of daily illumination	First day of test	First flowering	Height of plants	
				Measured on—	Average
					Inches
Short-day type:					
Tithonia tubaeformis	5 hours	June 19		Aug. 2	32
Do	8 hours	do	July 25	do	53
Do	10 hours	do	do	do	58
Do	12 hours	do	Aug. 22	do	53
Do	Darkened on alternate days	do	Sept. 8	do	32
Do	Darkened 10 a. m.–3 p. m	do	Nov. 10	do	33
Control	Full day	do	do	do	45
Helianthus angustifolius	5 hours	June 12	Aug. 1	do	22
Do	8 hours	do	July 18	do	23
Do	10 hours	do	July 24	do	27
Do	Darkened on alternate days	do	Aug. 14	do	19
Do	Darkened 10 a. m.–3 p. m	do	Oct. 9	do	13
Control	Full day	do	Oct. 7	do	16.5
Perilla frutescens	10 hours	June 29	July 24	July 24	15
Do	Darkened on alternate days	June 9	July 17	July 17	14.5
Do	Darkened 10 a. m.–3 p. m	do	Sept. 1		
Control	Full day	do	Sept. 2		
Giant Crimson Cosmos	Darkened on alternate days	do	Aug. 4	Aug. 4	18
Do	Darkened 10 a. m.–3 p. m	do	Oct. 7	Oct. 7	72
Control	Full day	do	Sept. 28	Sept. 28	84
Biloxi soybeans	Darkened on alternate days	June 8	Aug. 3	Aug. 3	12
Do	Darkened 10 a. m.–3 p. m	do	Sept. 18	Sept. 18	8
Control	Full day	do	Sept. 11	Sept. 11	35
Long-day type:					
Monarda didyma	10 hours	Mar. 9		June 21	15
Do	Darkened 10 a. m.–2 p. m	do	June 21	do	26
Control	Full day	do	June 15	June 15	36
Steironema ciliatum	10 hours	Mar. 25			
Do	Darkened 10 a. m.–2 p. m	do	June 20	June 20	35
Control	Full day	do	June 29	June 29	28

In order to obtain an indication of the effects of further shortening the light-and-darkness alternations, the group of plants listed in Table

1 was darkened each day from 10 a. m. to noon and again from 2 to 4 p. m. during the summer and early fall. The controls were exposed to the full day length. The date of first flowering and average height of plants are shown in Table 3.

TABLE 3.—*Time of flowering and growth of plants of the short-day, indifferent, and long-day types darkened from 10 a. m. till noon and again from 2 to 4 p. m. each day during the summer months, as compared with full-day illumination*

Type and species or variety	Treatment	First day of test	First flowering	Average height at flowering
Short-day type:				*Inches*
Peking soybeans	Darkened twice a day	June 29	Aug. 10	9
Control	Full-day illumination	do	do	12
Tokyo soybeans	Darkened twice a day	do	Aug. 30	22
Control	Full-day illumination	do	Aug. 17	21
Biloxi soybeans	Darkened twice a day	do	Sept. 23	11
Control	Full-day illumination	do	Sept. 9	28
Cosmos sulphureus	Darkened twice a day	June 24	Oct. 11	24
Control	Full-day illumination	do	Oct. 20	51
Perilla frutescens	Darkened twice a day	June 30	do	17
Control	Full-day illumination	do	Oct. 16	25
Indifferent type:				
Mandarin soybeans	Darkened twice a day	June 29	July 29	8
Control	Full-day illumination	do	July 28	9
Long-day type:				
Impatiens balsamina	Darkened twice a day	June 17	July 10	4
Control	Full-day illumination	do	do	9

EXPERIMENTS WITH ARTIFICIAL LIGHT

In the experiments with artificial light, a number of separate tests were first conducted in which all light treatments involved the same number of hours of total illumination daily, namely, 12 hours of light in the 24-hour cycle. Thus the total number of hours of illumination was the same as would be received by plants exposed to a 12-hour day. The first experiment in this group involved alternations of light and darkness of 6 hours in some instances and 4 hours in others, as compared with a 12-hour alternation. The long-day plant, *Rudbeckia bicolor* Nutt., and the Peking, Biloxi, and Mandarin varieties of soybeans were employed. The results are summarized in Table 4.

TABLE 4.—*Time of flowering and growth of plants exposed to 6-hour and 4-hour alternations of artificial light and darkness, as compared with 12-hour alternations*

Type and species or variety	Length of alternation	Duration of test	Time required for flowering	Average height at flowering
Long-day type:	*Hours*	*Days*	*Days*	*Inches*
Rudbeckia bicolor	6	51	34	33
Control	12	51	29	33
Indifferent type:				
Mandarin soybeans	6	51	34	45
Control	12	51	29	25
Mandarin soybeans	4	37	35	30
Control	12	37	21	24
Short-day type:				
Peking soybeans	6	51	(a)	42
Control	12	51	29	29
Peking soybeans	4	37	(a)	30
Control	12	37	21	20.5
Biloxi soybeans	6	51	(a)	40
Control	12	51	43	51

a Had not flowered at termination of experiment.

The next step in the investigations was the use of much shorter alternations of light and darkness, retaining the 12-hour alternation as a control. The three light intervals employed in addition to the control interval were 1 hour, 1 minute, and 15 seconds, respectively. In some of the tests continuous illumination also was used for the sake of comparison. In these experiments soybeans, Rudbeckia, Perilla, and Coleus were used. In some instances the green and the dry weights of the plants were obtained at the end of the test. Young seedlings of soybeans, Rudbeckia, and Perilla were employed, while for the Coleus cuttings that had already made considerable growth were used. This Coleus is a short-day type, flowering in winter. The results of the test are shown in Table 5. Under the shorter light intervals the soybeans presented a pale, etiolated appearance, and the stems were of a spindling, stringy type of growth, particularly under the 1-minute intervals. In Perilla the leaves showed the deepest purple color under continuous illumination. Under the 12-hour illumination the leaves were almost pure green in color, as is usually the case when the plants are in the flowering condition. The 1-hour light period gave larger, finer leaves than the 1-minute or the 15-second intervals. In Coleus the 12-hour light period gave normally colored, large, healthy leaves, while continuous light and the shorter intervals resulted in decided etiolation and poor coloration. Rudbeckia suffered a reduction in growth under the shorter light alternations, including the 1-hour period; and under the 1-minute exposure the young seedlings were unable to survive.

For the purpose of obtaining a more detailed picture of the comparative effects on plants of alternations of various short periods of light and darkness a series of eight artificially lighted compartments was brought into use. The 12-hour light period was retained as a control, and the experimental periods of light and darkness used in the remaining seven compartments were 5 seconds, 15 seconds, 1 minute, 5 minutes, 15 minutes, 30 minutes, and 1 hour, respectively. In this group of experiments the short-day type of plant was represented by *Cosmos sulphureus*, the long-day type by *Rudbeckia bicolor*, *Delphinium ajacis* L., *Althaea rosea* Cav., and *Beta vulgaris* L., while of two additional species that were included, *Fagopyrum vulgare* Hill and *Ipomoea batatas* (L.) Poir., the former may be classed as belonging to the indifferent type, and little is known as yet concerning the proper classification of the latter. The results of the tests with respect to time of flowering, height attained, and the green and dry weights for the several species are summarized in Table 6.

TABLE 5.—*Time of flowering and growth of plants exposed to equal alternations of artificial light and darkness of 15 seconds, 1 minute, and 1 hour, respectively, as compared with 12-hour alternations and continuous light*

Type and species or variety	Length of alternations	Duration of test	First day of test	Time required for flowering	Height of plants		Average green weight per plant			Average dry weight of 10 plants			
					Measured on—	Average	Entire plant	Tops	Roots	Entire plants	Tops	Roots	Ratio of tops to roots
Indifferent type:		*Days*		*Days*		*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
Mandarin soybeans	15 seconds	37	June 25		July 17	27							
Do	1 minute	37	do		do	27							
Do	1 hour	37	do		do	27							
Control	12 hours	37	do	21	do	27							
Short-day type:													
Biloxi soybeans	15 seconds	37	do		do	27							
Do	1 minute	37	do		do	27							
Do	1 hour	37	do		do	27							
Control	12 hours	37	do	34	do	27							
Biloxi soybeans	15 seconds	20	May 18		June 6	17.5	7.0	5.4	1.6	7.6	6.7	0.9	7.4
Do	1 minute	20	do		do	19	4.2	2.8	1.4	3.7	3.2	.5	6.4
Do	1 hour	20	do		do	16	12.4	7.8	4.6	12.5	10.0	2.5	4.0
Control	12 hours	20	do		do	13	11.2	7.0	4.2	11.8	8.9	2.9	3.1
Do	Continuous light	20	do		do	15	12.4	7.8	4.6	14.7	11.1	3.6	3.1
Perilla frutescens	15 seconds	43	June 7		July 20	26	31.2	23.5	7.7	41.0	33.2	7.8	4.3
Do	1 minute	43	do		do	21.5	21.9	17.8	4.1	26.3	22.0	4.3	5.1
Do	1 hour	43	do		do	18.5	40.2	30.9	9.3	53.0	42.8	10.2	4.2
Control	12 hours	43	do	32	do	37	58.7	49.8	8.9	69.3	58.8	10.5	5.6
Do	Continuous light	43	do		do	38	68.6	47.2	21.4	104.3	80.0	24.3	3.3
Coleus sp	15 seconds	43	do		do	9	41.5	36.4	5.1	27.8	23.3	4.5	5.2
Do	1 minute	43	do		do	9.5	48.0	43.8	4.2	28.5	25.0	3.5	7.1
Do	1 hour	43	do		do	10	46.4	42.3	4.1	31.8	27.5	4.3	6.4
Control	12 hours	43	do		do	24	75.0	60.0	15.0	50.8	45.5	5.3	8.6
Do	Continuous light	43	do		do	17	68.2	59.0	9.2	46.2	41.2	5.0	8.2
Long-day type:													
Rudbeckia bicolor	15 seconds	56	Mar. 23	37	May 18	20	14.0						
Do	1 minute	56	do	(a)									
Do	1 hour	56	do	33	May 18	20	16.8						
Control	12 hours	56	do	56	do	20	28.0						
Do	Continuous light	56	do	31	do	40	36.9						

[a] Died.

TABLE 6.—*Time of flowering and growth of plants exposed to equal alternations of artificial light and darkness of 5 seconds, 15 seconds, 1 minute, 15 minutes, 30 minutes, and 1 hour, respectively, as compared with 12-hour alternations*

Type and species or variety	Length of alternations	Duration of test	First day of test	Time required for flowering	Height of plants		Average green weight per plant			Average dry weight of 10 plants			
					Measured on—	Average	Entire plant	Tops	Roots	Entire plants	Tops	Roots	Ratio of tops to roots
Short-day type:		*Days*		*Days*		*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	
Cosmos sulphureus	5 seconds	42	Nov. 1		Dec. 10	31	16.0	14.8	1.2	14.3	13.0	1.3	10.0
Do	15 seconds	42	do		do	13	1.9	1.8	.1	1.5	1.4	.1	14.0
Do	1 minute	42	do		do	7	.5	.4	.1	.6	.5	.1	5.0
Do	5 minutes	42	do		do	8.5	1.1	1.0	.1	.9	.8	.1	8.0
Do	15 minutes	42	do		do	18	4.0	3.7	.3	3.3	3.0	.3	10.0
Do	30 minutes	42	do		do	20	3.3	3.1	.2	2.7	2.4	.3	8.0
Do	1 hour	42	do		do	22	11.0	10.0	1.0	9.9	9.0	.9	10.0
Control	12 hours	42	do		do	45	70.1	65.3	4.8	47.8	43.8	4.0	11.0
Long-day type:													
Rudbeckia bicolor	5 seconds	42	do	40	do	30	53.5	49.6	3.9	56.7	53.0	3.7	14.3
Do	15 seconds	42	do	40	do	25	30.5	26.8	3.7	38.7	35.0	3.0	11.7
Do	1 minute	42	do	40	do	17	15.0	14.0	1.0	17.1	15.5	1.6	9.7
Do	5 minutes	42	do	36	do	24	19.2	18.1	1.1	23.3	21.4	1.9	11.3
Do	15 minutes	42	do	36	do	28.5	27.3	25.1	2.2	29.6	26.6	3.0	8.9
Do	30 minutes	42	do	40	do	25	17.9	16.7	1.2	19.7	18.1	1.6	11.3
Do	1 hour	42	do	40	do	22	29.4	27.5	1.9	30.2	27.5	2.7	10.2
Control	12 hours	42	do		do	12	43.1	40.1	3.0	29.1	26.4	2.7	9.8
Delphinium ajacis	5 seconds	50	Dec. 13	37	Feb. 1	46	23.8	21.3	2.5	52.5	48.5	4.0	12.1
Do	15 seconds	50	do	37	do	40	20.0	17.8	2.2	44.8	41.8	3.0	13.9
Do	1 minute	50	do	41	do	33	19.0	17.5	1.5	43.8	40.8	3.0	13.6
Do	5 minutes	50	do	45	do	23	10.0	9.0	1.0	24.0	21.0	3.0	7.0
Do	15 minutes	50	do	41	do	27	11.5	10.2	1.3	27.5	25.0	2.5	10.0
Do	30 minutes	50	do	42	do	33.5	18.3	16.5	1.8	38.0	35.5	2.5	14.2
Do	1 hour	50	do	48	do	39	25.8	22.5	3.3	52.5	47.0	5.5	8.5
Control	12 hours	50	do	41	do	42	28.9	25.6	3.3	52.3	47.3	5.0	9.4
Althaea rosea	5 seconds	50	do		do	31	103.0	88.0	15.0	130.0	97.0	33.0	2.9
Do	15 seconds	50	do		do	30	85.7	76.3	9.4	95.6	78.3	17.3	4.5
Do	1 minute	50	do		do	15	33.0	29.0	4.0	34.3	28.0	6.3	4.4
Do	5 minutes	50	do		do	11.5	34.0	31.0	3.0	38.0	31.0	7.0	4.4
Do	15 minutes	50	do		do	20	65.7	58.0	7.7	68.3	57.7	10.6	5.4
Do	30 minutes	50	do		do	27	97.3	84.3	13.0	112.0	90.0	22.0	4.1
Do	1 hour	50	do		do	23	72.0	63.0	9.0	86.0	68.3	17.7	3.5
Control	12 hours	50	do		do	28	146.0	123.0	23.0	190.0	131.0	59.0	2.2
Beta vulgaris	5 seconds	75	Mar. 22		June 5	[a] 18	77.5	38.4	39.1	88.8	37.0	51.8	.7
Do	15 seconds	75	do		do	[a] 15	29.8	16.6	13.2	35.3	19.1	16.2	1.2
Do	1 minute	75	do		do	[a] 16	49.8	32.6	17.2	48.9	29.0	19.9	1.4
Do	5 minutes	75	do	51	do	[a] 20	68.5	49.5	19.0	70.0	42.9	27.1	1.5
Do	15 minutes	75	do		do	[a] 8	6.4	5.6	.8	3.7	3.6	.1	36.0

Do	30 minutes	75	...do...		...do...	[a] 11	18.6	13.9	4.7	15.5	10.9	4.6	2.4
Do	1 hour	75	...do...		...do...	[a] 14	41.0	25.4	15.6	44.0	23.4	20.6	1.1
Control	12 hours	75	...do...		...do...	[a] 17	68.0	31.1	36.9	94.6	33.2	61.4	.5
Indifferent type:													
Fagopyrum vulgare	5 seconds	34	Feb. 7	24	Mar. 12	58	45.2	44.2	1.0	42.4	40.7	1.7	23.9
Do	15 seconds	34	...do...	34	...do...	43	18.5	18.2	.3	14.8	14.3	.5	28.6
Do	1 minute	34	...do...	23	...do...	36	12.0	11.6	.4	8.8	8.2	.6	13.7
Do	5 minutes	34	...do...	24	...do...	38	20.0	19.5	.5	16.0	15.2	.8	19.0
Do	15 minutes	34	...do...	29	...do...	28	10.6	10.3	.3	7.9	7.6	.3	25.3
Do	30 minutes	34	...do...	24	...do...	33	15.5	15.1	.4	11.1	10.6	.5	21.2
Do	1 hour	34	...do...	29	...do...	40	20.6	19.7	.9	15.5	14.4	1.1	13.1
Control	12 hours	34	...do...	21	...do...	54		32.3		31.1	29.4	1.7	17.3
Type undetermined:													
Ipomoea batatas	5 seconds	98	Feb. 3		May 11	49	248.0	146.0	102.0	[b] 644.0	122.0	26.0	.23
Do	15 seconds	98	...do...		...do...	49	201.0	140.0	61.0	[b] 434.0	124.0	16.0	.40
Do	1 minute	98	...do...		...do...	52	156.0	135.0	21.0	[b] 136.0	112.0	13.0	4.7
Do	5 minutes	98	...do...		...do...	60	105.0	92.5	12.5	76.5	69.5	7.0	9.9
Do	15 minutes	98	...do...		...do...	35	18.0	12.5	5.5	20.5	16.0	4.5	3.6
Do	30 minutes	98	...do...		...do...	32	32.0	27.0	5.0	37.0	32.0	5.0	6.4
Do	1 hour	98	...do...		...do...	60	43.0	35.0	8.0	48.5	44.0	4.5	9.8
Control	12 hours	98	...do...		...do...	62	352.0	221.0	131.0	[b] 931.0	179.0	32.0	.24

[a] Length of longest leaves.

[b] Includes weights of "tubers," which are not included in the column "roots."

The Rudbeckia seedlings used were somewhat older than those employed in the previous experiment, and the flowering stage was reached under all the short alternations. Under the light periods ranging from 1 minute to 30 minutes the plants were chlorotic, but under all other light treatments they showed the normal dark-green color. In Cosmos the same general relations existed, the etiolation being most marked under the 1-minute, 5-minute, and 15-minute light intervals, and the normal color showing under the 5-second, 1-hour, and 12-hour intervals. Delphinium seemed to be most adversely affected by the 5-minute light interval, and under this period only one plant survived. First-year seedlings of *Althaea rosea* were used in the test, and none flowered or developed flowering stems although the leaves developed abnormally long petioles. The 5-second and 12-hour plants showed the darkest green color, the 15-second and 1-hour individuals being next, and the others showing a decidedly lighter, yellowish green color. Only one seedling survived under the 5-minute light interval. For the experiment with the table beet (*Beta vulgaris*) young seedlings of the Early Eclipse variety were used. A single plant under the 1-minute light interval flowered, and one plant developed a stem under the 15-second light period. The heights recorded in Table 6 refer to the lengths of the longest leaves. The smallest plants, with leaves of poorest color, were those under the 15-minute light interval. The buckwheat (*Fagopyrum vulgare*) showed most etiolation under the 15-minute light interval, with progressive improvement in the green color under both longer and shorter intervals. In the sweetpotato (*Ipomoea batatas*) the foliage was yellow under the 15-minute, 30-minute, and 1-hour intervals, and no tubers or thickened roots were formed under these exposures or under the 5-minute interval. The best development of tubers occurred under the 12-hour and the 5-second intervals.

In all the foregoing experiments with artificial light the total number of hours of light and darkness received daily by the plants was the same. In the following series of tests the same plants and equipment were used, but the intervals of light and darkness were of unequal value, although the complete cycles remained the same as in the preceding experiments. In the first group of tests the interval of light was just half that of darkness in each case. For example, instead of the previous alternation of 5 seconds of light and 5 seconds of darkness, the new cycle consisted of 3⅓ seconds of light and 6⅔ seconds of darkness; and in place of 1 hour each of light and of darkness, the new cycle was composed of 40 minutes of light and 80 minutes of darkness. In all cases the total daily illumination was 8 hours. As a control, an 8-hour light period was employed. Biloxi soybeans, *Cosmos sulphureus*, and *Rudbeckia bicolor* were used for the tests. The soybeans did not long survive the unfavorable effects of the ⅓-minute, 3⅓-minute, and 10-minute light intervals, and the Cosmos soon perished under these intervals and also under the 10-second and 20-minute intervals, so that data on comparative weights of the plants could not be obtained. Under these intervals the heights of the soybeans and Cosmos recorded were those reached at the time of death, while in other cases the heights attained at the end of the experiment are reported. The results of the tests are summarized in Table 7. None of the soybeans presented a healthy appearance

except those exposed to the 8-hour light period, and Cosmos also showed unfavorable effects from the shorter light intervals. Rudbeckia showed a more or less normal green color under the 3½-second, 10-second, and 8-hour light periods.

In the final series of experiments the durations of the intervals used in the preceding tests were reversed, so that in each complete cycle the light interval was double the darkness interval, and the total daily illumination was 16 hours in each case. In the first experiment Rudbeckia and Cosmos were used. In this experiment an installation for automatic control of temperature and relative humidity was employed. A current of conditioned air sufficiently rapid to insure substantially the same temperature and humidity in each compartment was forced through the light compartments. On four unusually warm days the midday temperatures reached 75° to 80° F. and on a single day 90°. At other times the temperature was held at $71° \pm 2°$ and the relative humidity was maintained at 53 ± 2 per cent. The weekly mean temperature ranged from 71° to 75° during the period of the test. In the early morning of the thirty-fifth day there was a breakdown in the control system that caused the temperature to rise above 100° for a few hours. It was therefore decided to discontinue the experiment. The Cosmos plants were so badly dried out by the excessive heat that satisfactory data on weights of the plants could not be obtained. The Rudbeckia, however, showed no sign of material injury.

The increased illumination produced obvious improvement in nutrition and growth under all treatments, as compared with the results in the preceding experiment; but the contrasts in condition of the plants under the different light treatments were more or less similar to those in the preceding experiment. The Rudbeckia plants had not flowered when the test was interrupted, but all had formed flower buds. None of the Cosmos plants showed flower buds. The results of the experiment are summarized in Table 8. A similar experiment with Mandarin and Peking soybeans is also shown in Table 8. In this case an effort was made to maintain the temperature at 77° F. and the relative humidity at 55 per cent so far as weather conditions would permit. As it happened, the weather was unseasonably warm for a considerable portion of the time so that the temperature was beyond control, and toward the end of the experiment it became necessary to cut off all artificial heat.

TABLE 7.—*Time of flowering and growth of plants exposed to various short alternations of artificial light and darkness in a ratio of 1 to 2, as compared with alternations of 12 hours each of light and darkness or 8 hours of light and 16 hours of darkness*

Type and species or variety	Length of alternations		Duration of test	Time required for flowering	Average height of plants	Average green weight per plant			Average dry weight of 10 plants			Ratio of tops to roots
	Light	Darkness				Entire plant	Tops	Roots	Entire plants	Tops	Roots	
Short-day type:			*Days*	*Days*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Biloxi soybeans	3⅓ seconds	6⅔ seconds	41		30							
Do	10 seconds	20 seconds	41		25							
Do	⅔ minute	1⅓ minutes	41		14							
Do	3⅓ minutes	6⅔ minutes	41		19							
Do	10 minutes	20 minutes	41		17.5							
Do	20 minutes	40 minutes	41		25							
Do	⅔ hour	1⅓ hours	41		30							
Control	12 hours	12 hours	41	36	32							
Cosmos sulphureus	3⅓ seconds	6⅔ seconds	39		13							
Do	10 seconds	20 seconds	39		5							
Do	⅔ minute	1⅓ minutes	39		5							
Do	3⅓ minutes	6⅔ minutes	39		6.5							
Do	10 minutes	20 minutes	39		5							
Do	20 minutes	40 minutes	39		7							
Do	⅔ hour	1⅓ hours	39		13							
Control	8 hours	16 hours	39		32							
Long-day type:												
Rudbeckia bicolor	3⅓ seconds	6⅔ seconds	50	35	32	9.7	9.2	0.5	9.3	8.8	0.5	17.6
Do	10 seconds	20 seconds	50	35	28.5	7.7	7.3	.4	8.0	7.6	.4	19.0
Do	⅔ minute	1⅓ minutes	50	38	18	4.3	4.1	.2	5.8	5.6	.2	28.0
Do	3⅓ minutes	6⅔ minutes	50	40	18.5	4.8	4.6	.2	5.6	5.3	.3	17.7
Do	10 minutes	20 minutes	50	42	21	5.0	4.8	.2	3.8	3.6	.2	18.0
Do	20 minutes	40 minutes	50	45	26	6.4	6.1	.3	5.0	4.6	.4	11.5
Do	⅔ hour	1⅓ hours	50	49	17	7.9	7.5	.4	6.3	5.9	.4	14.8
Control	8 hours	16 hours	50		8	30.0	26.3	3.7	28.1	24.5	3.6	6.8

TABLE 8.—*Time of flowering and growth of plants exposed to various short alternations of artificial light and darkness in a ratio of 2 to 1, as compared with an alternation of 16 hours of light and 8 hours of darkness*

Type and species or variety	Length of alternations		Duration of test	Time required for flowering	Average height of plants	Average green weight per plant			Average dry weight of 10 plants		
	Light	Darkness				Entire plant	Tops	Roots	Entire plants	Tops	Roots
			Days	*Days*	*Inches*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Short-day type:											
Cosmos sulphureus	6⅔ seconds	3⅓ seconds	36		25						
Do	20 seconds	10 seconds	36		21						
Do	1⅓ minutes	⅔ minute	36		14.5						
Do	6⅔ minutes	3⅓ minutes	36		9						
Do	20 minutes	10 minutes	36		14						
Do	40 minutes	20 minutes	36		18						
Do	1⅓ hours	⅔ hour	36		22						
Control	16 hours	8 hours	36		23						
Peking soybeans	6⅔ seconds	3⅓ seconds	42		50	8.1	7.5	0.6	13.5	12.8	0.7
Do	20 seconds	10 seconds	42		50	10.4	9.6	.8	12.7	11.9	.8
Do	1⅓ minutes	⅔ minute	42		50	15.3	14.3	1.0	20.9	19.9	1.0
Do	6⅔ minutes	3⅓ minutes	42		50	10.2	9.1	1.1	13.7	12.9	.8
Do	20 minutes	10 minutes	42		50	9.2	8.5	.7	12.7	11.8	.9
Do	40 minutes	20 minutes	42		50	8.2	7.6	.6	11.7	11.1	.6
Do	1⅓ hours	⅔ hour	42		42	11.6	10.7	.9	16.2	15.1	1.1
Control	16 hours	8 hours	42		44	12.4	11.6	.8	15.1	14.0	1.1
Long-day type:											
Rudbeckia bicolor	6⅔ seconds	3⅓ seconds	36	* 35	25	23.2	22.0	1.2	21.4	19.9	1.5
Do	20 seconds	10 seconds	36	* 35	21	16.1	15.0	1.1	17.1	15.7	1.4
Do	1⅓ minutes	⅔ minute	36	* 35	20	18.1	16.8	1.3	14.4	12.9	1.5
Do	6⅔ minutes	3⅓ minutes	36	* 35	14.5	14.0	12.3	1.7	15.0	13.0	2.0
Do	20 minutes	10 minutes	36	* 35	18	16.3	14.7	1.6	16.6	14.7	1.9
Do	40 minutes	20 minutes	36	* 35	22	15.9	14.8	1.1	16.9	14.9	2.0
Do	1⅓ hours	⅔ hour	36	* 35	22	29.9	27.6	2.3	30.4	27.1	3.3
Control	16 hours	8 hours	36	* 35	23	25.4	23.7	1.7	18.9	17.0	1.9
ndifferent type:											
Mandarin soybeans	6⅔ seconds	3⅓ seconds	42		50	12.7	11.7	1.0	15.7	14.9	.8
Do	20 seconds	10 seconds	42		50	17.1	16.1	1.0	23.8	22.6	1.2
Do	1⅓ minutes	⅔ minute	42		50	13.5	12.3	1.2	17.1	15.8	1.3
Do	6⅔ minutes	3⅓ minutes	42		50	13.9	13.0	.9	18.7	17.8	.9
Do	20 minutes	10 minutes	42		50	14.4	13.4	1.0	22.8	21.7	1.1
Do	40 minutes	20 minutes	42		50	12.6	11.9	.7	16.9	16.1	.8
Do	1⅓ hours	⅔ hour	42		46	15.4	14.4	1.0	20.5	19.6	.9
Control	16 hours	8 hours	42	32	50	22.9	21.6	1.3	35.2	33.0	2.2

* Flower buds were present but none had opened.

A summary of the thermographic records obtained in the light chambers during the experiments is given in Table 9. The data include the maximum and minimum temperatures recorded in each series; also the mean maximum, the mean minimum, and the approximate mean or average of the latter two values.

TABLE 9.—*Temperature in light-proof chambers used in experiments summarized in Tables 5 to 8*

Table No. and plant	Temperature of light chambers (° F.)				
	Maximum	Minimum	Mean maximum	Mean minimum	Mean of mean maximum and mean minimum
Table 5:					
Mandarin and Biloxi soybeans	88	64	82	71	76
Rudbeckia	99	69	88	72	80
Biloxi soybeans	84	65	80	67	74
Perilla and Coleus	86	66	84	70	77
Table 6:					
Rudbeckia and Cosmos	81	65	75	69	72
Delphinium and Althaea	77	64	72	68	70
Fagopyrum	79	65	74	69	72
Beta	80	60	73	67	70
Ipomoea	80	62	74	67	71
Table 7:					
Biloxi soybeans and Cosmos	85	70	83	76	80
Rudbeckia	87	61	78	71	75
Table 8:					
Rudbeckia and Cosmos	90	69	74	71	73
Mandarin and Peking soybeans	99	62	82	73	78

DISCUSSION OF RESULTS

In nature, plants are normally exposed to a 24-hour cycle of day and night, there being, of course, only one period of light and one of darkness in the cycle. Under these circumstances the variation in relative length of day and night dependent upon latitude and season may profoundly affect the development of the plant. By excluding the daylight of early morning or late afternoon so as to shorten the daily light period, on the one hand, and by using artificial light to prolong the daily illumination, on the other hand, the distinctive effects of short days and long days on flowering and fruiting and other features of growth may be reproduced, under suitable conditions, in any latitude and at any season. Results of the experiments presented in this paper, however, indicate that different variations from the 24-hour cycle of light and darkness may produce radically different effects, even though the total number of hours of illumination is the same.

In previous experiments it has been shown that diminishing the number of hours of the midsummer daylight to which plants are continuously exposed hastens the flowering of short-day plants and retards that of long-day plants, whereas an equal period of darkening in the middle of the day, by which the short day is divided into two parts, has little or no effect on the time of flowering.

Table 1 summarizes the results of an experiment in which short-day and long-day plants were subjected on alternate days to a full day and to a 10-hour day of sunlight. These data show that under the conditions that prevailed the interposition of the long day did

not nullify the hastening effect of the short day on the flowering of the short-day plants, while it did very nearly prevent the retarding action on the long-day plants. Reduction in height attained was associated with early flowering under the long-day and short-day alternations.

When the 24-hour cycle of day and night was replaced by a 48-hour cycle (illumination on alternate days, see Table 2), consisting of about 15 hours of light and 33 hours of darkness, there was a pronounced hastening action on flowering in five typical short-day plants. The short-day effect, therefore, is still in evidence, although comparison with the results of 8, 10, and 12 hours of light daily shows that the hastening effect has been much reduced in Tithonia and Helianthus.

The present data, however, bear chiefly on the effects of cycles of light and darkness much shorter than the 24-hour cycle provided

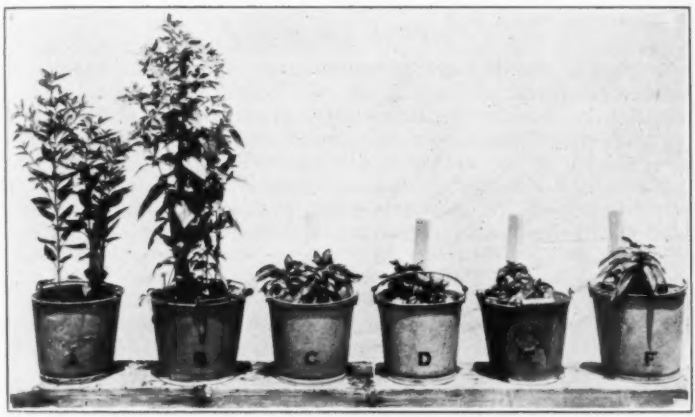

FIGURE 1.—Fringed loosestrife (*Steironema ciliatum* (L.) Raf.), a long-day plant, exposed to the full day length of late spring and early summer and to regulated daily light periods, beginning March 25. The illumination conditions were: A, Full day; B, darkened 10 a. m. to 2 p. m.; C, 12-hour day; D, 10-hour day; E, 8-hour day; F, 5-hour day. Midday darkening (B) failed to suppress flowering, the usual effect of the short day (C–F)

by nature. The results in Table 2 bring out the remarkable fact that darkening during the middle of the day for as long as 5 hours has either no effect at all or only a slight effect on the time of flowering as compared with the full day length of summer. This is in sharp contrast with 8, 10, or 12 hours of uninterrupted daily illumination. In earlier experiments much the same results were obtained by a 2-hour midday darkening. All short-day plants studied have failed to show response to midday darkening with respect to flowering; that is, they have behaved about the same as when exposed to the full day length. The majority though not all of the long-day plants tested also have failed to respond to the midday darkening.

The ineffectiveness of midday darkening in delaying elongation of flowering stems in Steironema is shown in Figure 1. It will be observed that the effect of this treatment was to break up the normal 24-hour cycle, for in each 24-hour period the plants were subjected to two intervals of light and two intervals of darkness. The Stei-

ronema plants exposed to the full day length, beginning March 25, began flowering June 29; those darkened in the middle of the day began flowering nine days earlier; all other treatments suppressed flowering. Midday darkening, even though prolonged, as a rule does not produce the usual effects of a short day on either the long-day or the short-day type of plant.

Further evidence that relatively short cycles of light and darkness tend to produce effects similar to those of a long day or of continuous light in that they favor flowering in long-day plants but not in short-day plants is furnished by the results shown in Table 3. Three daily light periods, aggregating about 10 hours in duration, failed to materially change the date of flowering in the long-day or indifferent types, and in the short-day plants either failed to change or actually delayed the date of flowering, as compared with the effect of the full summer-day length. With one exception, the darkening had the effect of reducing the heights of the plants.

EFFECTS ON FLOWERING

As regards the normal 24-hour cycle, an even division into 12 hours of daylight and 12 hours of darkness permits of eventual flowering and fruiting in the majority of plants of both the long-day and short-day types. As a rule, however, the 12-hour day is above the optimum for flowering in the short-day group and below the optimum for the long-day group. In studying with artificial illumination the effects of relatively short cycles involving an even distribution of light and darkness, the 12-hour day naturally was employed as the control. In the long-day plant Rudbeckia, a 6-hour alternation of light and darkness somewhat hastened the appearance of blossoms. (Table 4.) In the short-day varieties of soybeans, flowering was decidedly delayed by 6-hour and 4-hour alternations. The effects on height attained by the plants were somewhat variable.

Results obtained with alternations of light and darkness of 1 hour or less soon showed that a distinction must be drawn between effects on reproduction and those on growth and general nutrition of the plant. In the experiments with alternations of light and darkness of 1 hour, 1 minute, and 15 seconds (Table 5) and those including alternations of 1 hour, 30 minutes, 15 minutes, 5 minutes, 1 minute, 15 seconds, and 5 seconds (Table 6), it will be observed that flowering did not take place in the short-day plants—soybeans, Perilla, Coleus and Cosmos—under any of these exposures, the effect being that of a long day or continuous light. Under a 12-hour day all flowered except Coleus and Cosmos, and these had formed flower buds when the experiment was stopped. Of the long-day plants used, it is evident that Rudbeckia flowered much earlier under all the short alternations than under the 12-hour day, and about the same time as under continuous illumination. Delphinium, a less pronounced long-day plant, flowered about the same time under all of the light periods. The first-year plants of Althaea, another long-day type, did not flower under any of the alternations. The beet (Beta) is a long-day plant and when exposed to a long day and a cool temperature may behave as an annual. Under the warm temperature of the present experiment a single plant flowered with the

1-minute alternations, and a single seed stalk was formed with the 15-second alternations, although it did not flower. These results show a tendency of the plants under the short alternations to behave as when exposed to a long day. Buckwheat (Fagopyrum), which is relatively indifferent to changes in day length with respect to flowering, apparently was slightly delayed in flowering by the short alternations. The sweetpotato (Ipomoea) is ordinarily a nonflowering type of plant at the higher latitudes, and failed to blossom in the present experiment.

With the cycles remaining the same as in the preceding tests but with the light periods having one-half the value of the periods of darkness (Table 7), Rudbeckia still flowered under all the short alternations; but flowering was progressively delayed with increase in the length of the alternations. Biloxi soybeans again failed to flower under the short alternations. After 39 days Cosmos was showing flower buds only under the 8-hour day. Therefore, even with a total of only 8 hours of illumination daily, the effect on flowering of the short alternations is essentially that of a long day or continuous light. Finally, with the same cycles as before but with the light periods double the periods of darkness (Table 8), Rudbeckia readily developed flower buds. At the end of 36 days Cosmos had formed no flower buds, and neither the Peking nor Mandarin soybeans had flowered. The Peking variety of soybeans is a short-day type, while the Mandarin behaves as such only in regions having a summer day length of more than 17 hours. Here, again, with total daily illumination of 16 hours, the effects of the short alternations are those of a long day or continuous light on both the long-day and the short-day type of plant.

EFFECTS ON GROWTH AND GENERAL NUTRITION

In the experiments with short alternations of light and darkness of equal duration it was quickly observed that pathological symptoms are apt to develop and that the severity of the symptoms depends on the particular alternations employed. In soybeans it was found that the leaves are most severely affected by the 1-minute intervals of light and darkness. The leaves may be much reduced in size, assume a pale yellowish green color, and soon develop numerous areas of dead tissue. (Fig. 2.) Similar though less pronounced effects were produced by other alternations, ranging from 30 minutes to 15 seconds, but the leaves developed normally when the alternations of light and darkness were reduced to 5 seconds. Other plants also showed more or less severe pathological symptoms in the leaves when exposed to the light intervals of 1 minute or to those ranging from 15 seconds to 15 minutes, although the symptoms were not always so clearly defined as in soybeans. In some instances young seedlings of Rudbeckia and other plants were unable to survive under the 1-minute light intervals. Destruction of chlorophyll seems to be an important feature in the unfavorable effects of these particular alternations of light and darkness. With change in the length of the light interval in either direction there was definite improvement in the appearance of the foliage leaves. With a decrease to 5 seconds or an increase to 30 minutes or an hour in the duration of the intervals, the plants usually became normal in color and gen-

eral appearance, except sweetpotatoes, which were still chlorotic under the 1-hour intervals.

Attenuation was another outstanding feature in the unfavorable effects of the intermediate intervals of light and darkness. In soybeans the stems were not necessarily shortened, but they were weak and much reduced in size, thus tending to become viny. In most other species, also, the stems seemed to be abnormally small in comparison with the height, as if there were a stimulus toward stem elongation out of proportion to the basal nutrition of the plant.

The results shown in Tables 5 and 6 make it clear that, except in soybeans, the heights attained by the plants usually were reduced by the alternations ranging from 15 seconds to 30 minutes or an hour.

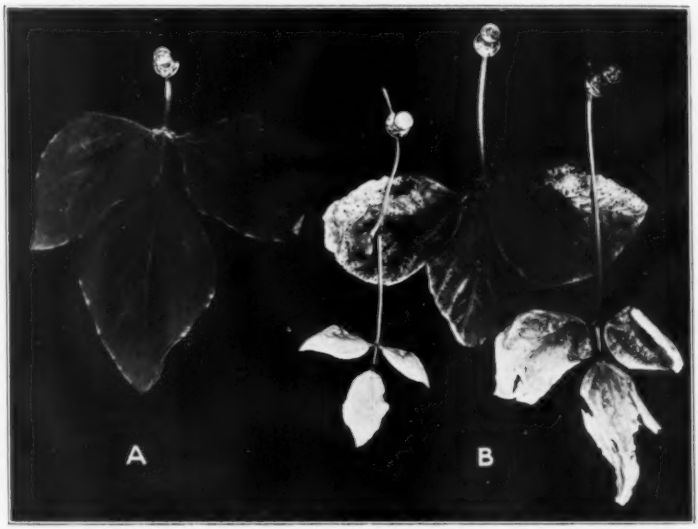

FIGURE 2.—Leaves of soybeans (*Soja max* (L.) Piper) grown with artificial illumination: A, Leaves of plants exposed to 12-hour alternations of light and darkness, normal in color and general development; B, leaves of plants exposed to 1-minute intervals of light and darkness, markedly chlorotic and much reduced in size, containing numerous spots of dead tissue, and showing a tendency to die prematurely

It is quite clear, also, that the 5-second alternation generally gave increases in height almost or quite equal to those produced by the relatively long alternations used as controls. In the long-day plant Rudbeckia, the 12-hour day retarded elongation of the flowering stem, as would be expected, and some indication of this effect persisted in the 1-hour alternation. The comparative effects of the different alternations on the height attained, as well as on flowering and degree of attenuation, in Cosmos and Delphinium are well shown in Figures 3 and 4. It is seen that in the former there is a progressive decrease in stem elongation and in size or mass of the plants from the 12-hour alternations down to the 1-minute periods of light and darkness, and a corresponding increase in elongation and stockiness as the periods are further shortened to five seconds. In Delphinium the same relations hold, except that the maximum depressing effects are produced

with the 5-minute intervals and there is even greater improvement in growth with the 5-second alternations. In the short-day plant Cosmos, flower buds formed only under the 12-hour intervals of light and darkness; whereas in the long-day type Delphinium, flowering occurred with equal readiness under all alternations employed.

Total growth, as measured by green weights and oven-dried weights of the entire plants, shows much the same effects from the different alternations of light and darkness as does the height attained by the plants. In nearly all cases maximum depressing effects on green and dry weights of the plants are associated with maximum depression in height. In practically every instance the minimum green weight

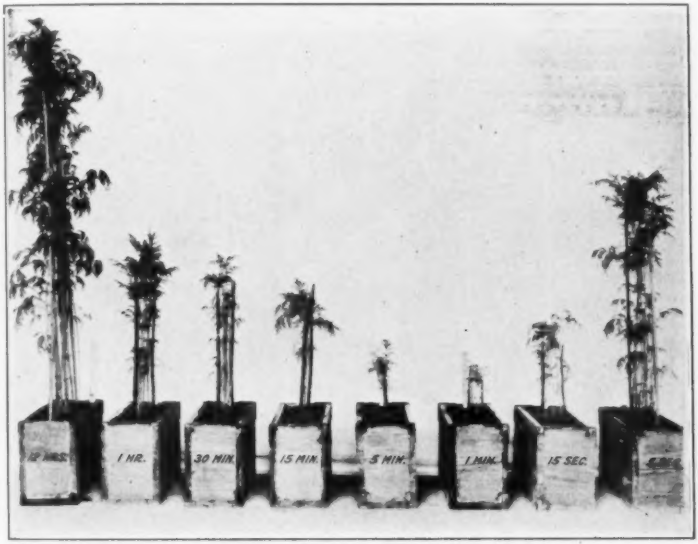

FIGURE 3.—Yellow cosmos (*Cosmos sulphureus* Cav.), a short-day plant, grown with equal alternations of light and darkness ranging from 12 hours to 5 seconds. With decrease in the intervals of light and darkness there is progressive decrease in height, size, and weight of the plants (see Table 6) and increase in etiolation and attenuation till the 1-minute intervals are reached. Further shortening of alternations causes marked improvement in growth and appearance of the plants. All intervals from 1 hour downward are almost equally unfavorable for flowering

is associated with minimum dry weight. The indicated values for water content of the green plants can be regarded as only approximations and do not show any very definite contrasts under the different alternations, except that in several instances the water content seems to be relatively high under the 12-hour alternations. Data were collected on the relative weights of tops and roots under the different treatments, and values for the ratio of tops to roots are included in the tables. The values for weights of roots are not accurate, however, because of difficulties in recovering all roots from the soil, and no great importance can be attached to the results. In the case of Ipomoea no tubers were formed under the alternations ranging from five minutes to one hour. To obtain some indication as to whether failure in absorption of soil nutrients could be responsible for the unfavorable

results with the intermediate short alternations, determinations of total ash in the tops of Delphinium and Fagopyrum (Table 6) were made by C. W. Bacon, of the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry. With alternating periods of light and darkness of 12 hours, 5 minutes, and 5 seconds, the corresponding percentages of total ash in Delphinium were 12.15, 13.64, and 10.49, respectively; in Fagopyrum, alternations of 12 hours, 15 minutes, and 5 seconds gave percentages of 14.97, 18.80, and 13.77, respectively. These data give no indication of deficiency in ash constituents under the harmful alternations of light and darkness.

When the light periods were reduced to one-half the periods of darkness, without change in duration of the total cycles, the unfavor-

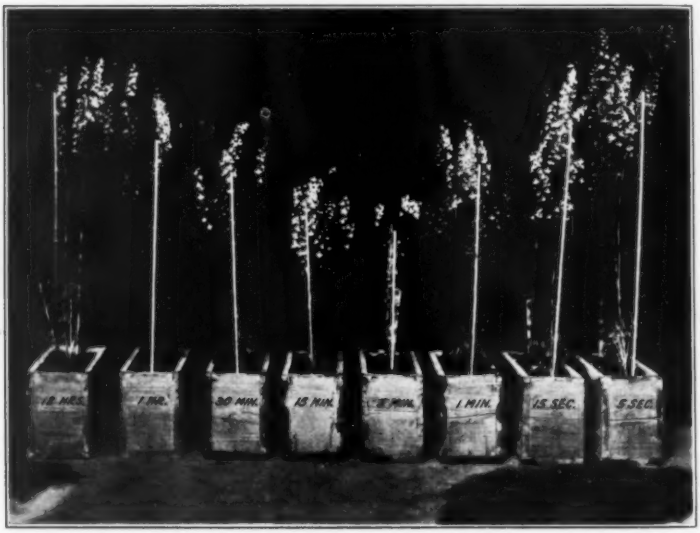

FIGURE 4.—Rocket larkspur (*Delphinium ajacis* L.), a long-day plant, grown with equal alternations of light and darkness ranging from 12 hours to 5 seconds. The comparative effects of the different alternations on nutrition and growth are about the same for long-day as for short-day plants. (Fig. 3.) As regards flowering, however, it is apparent that none of the shorter alternations show a retarding action, whereas all have this effect on short-day plants

able effects on growth of the cycles ranging from 30 seconds to 1 hour were accentuated. (Table 7.) Cosmos soon died when exposed to these alternations, and soybeans were able to survive only under the 30-second and 1-hour cycles, so that satisfactory data on green and dry weights could not be obtained. Rudbeckia survived under all treatments, and the effects on growth of reducing the light periods to one-half the periods of darkness, as compared with equal alternations of light and darkness in the corresponding cycles, are indicated in Figures 5 and 6. The relative effects of the different cycles on growth are approximately the same in the two cases, but it is obvious that reduction in the ratio of light to darkness in each cycle has greatly reduced the vigor and the amount of growth. This is clearly shown also in the green and dry weights recorded in Tables 6 and 7. The

retarding action on stem elongation of the 12-hour alternation decreases progressively in the next three shorter alternations (fig. 5), and the retarding action of the 8-hour light period decreases in the next two shorter alternations (fig. 6).

FIGURE 5.—Pinewoods coneflower (*Rudbeckia bicolor* Nutt.), a more pronounced long-day plant than Delphinium (fig. 4), grown with equal alternations of light and darkness ranging from 12 hours to 5 seconds. The relative effects of the different alternations on growth and on flowering resemble those in Delphinium, except that the 12-hour light period retards stem elongation and flowering, and these effects still persist in decreasing degree with the 1-hour and the 30-minute periods. In this case maximum growth was obtained with the 5-second alternations. (Table 6)

When the light period was increased to twice the period of darkness in each of the cycles, there was decided improvement in the vigor and general nutrition of the plants. (Table 8.) In Rudbeckia and

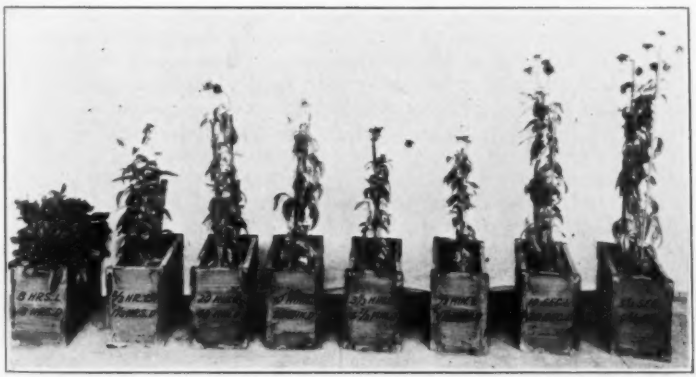

FIGURE 6.—Pinewoods coneflower grown with the same total cycles of light and darkness as those applied to the plants shown in Figure 5, but with the light periods in each cycle only one-half as long as the periods of darkness. Thus the plants in all cases received a total of 8 hours of light daily instead of 12 hours. There was no fundamental change in relative effects of the different cycles on growth and flowering, but comparison with Figure 5 shows there was a decided reduction in growth of the plants in all cases

Cosmos the same differential effects of the shorter alternations previously noted were still in evidence with respect to both height and weight in the former and height in the latter plants. With soybeans,

however, the increased ratio of light to darkness seemed largely to overcome the adverse effects of the alternations ranging from 15 seconds to 30 minutes. The heights attained by Rudbeckia and Cosmos are comparable to those produced with an even ratio of light and darkness, except under the 16-hour as compared with the 12-hour light period. In Rudbeckia the increase in the ratio of light to darkness failed to increase the weights of the plants.

The results presented in this paper relate primarily to effects of relatively short alternations of light and darkness ranging from 6 hours to 5 seconds, with a 12-hour alternation of light and darkness as the principal basis for comparison. As already pointed out, the 12-hour alternation, which supplies the only equal distribution of light and darkness occurring in the normal 24-hour cycle, is not optimum for flowering in most plants of either the long-day or the short-day type. A light-and-darkness ratio of 5:7, as supplied in a 10-hour day, or a ratio of 2:1, as in an 8-hour day, is more likely to be optimum for most short-day plants; and the reverse ratios of 7:5 and 2:1, as in day lengths of 14 and 16 hours, respectively, are more nearly optimum for typical long-day plants. It will be of considerable interest in this connection to study the effects of 10-hour and 8-hour alternations having equal periods of light and darkness, and other alternations between those of 12 hours and 6 hours with both equal and unequal periods of light and darkness, which do not occur in the natural 24-hour cycle. It is hoped that these studies can be carried out with rather rigid control of temperature and other environmental factors.

SUMMARY

Breaking the continuity of the daily illumination period of plants by darkening them in the middle of the day for periods of 1 or 2 hours to as long as 5 hours may materially affect the general nutrition and amount of growth, but as a rule it fails to influence reproductive activities to a degree at all comparable with that produced by excluding the early morning or late afternoon light of the long summer days. This applies to all short-day plants that have been studied and to a majority of long-day plants, and means that reducing the hours of daily illumination by thus breaking the continuity of the illumination is not generally effective in inducing reproductive activity in short-day plants or in interfering with initiation of the process in long-day plants. The effects are essentially those of the long day.

Further breaking up of the daylight period by darkening the plants from 10 a. m. to noon and from 2 to 4 p. m. seemed to accentuate the lack of effectiveness of this method of reducing the hours of illumination in bringing about the characteristic effects of a short-day length. In some short-day plants flowering was actually delayed as compared with results under the full day of summer.

When the test plants were completely darkened on alternate days during the summer months, thus substituting a 48-hour cycle of light and darkness for the normal 24-hour cycle and furnishing about 15 hours of light and 33 hours of darkness in the cycle (corresponding to 7½ hours of light daily), the effects were those of a short day, although these effects were much weaker than those of a normal 8-hour or 10-hour day.

Exposing a group of short-day plants alternately to a 10-hour day and to the full day length of summer produced an effect intermediate between the effects of a long day and a short day as regards initiation of flowering. *Impatiens balsamina* (garden balsam), the only long-day plant tested, behaved as when exposed daily to a long day.

Following the experiments in which sunlight was the source of illumination, a number of species were grown under a series of different, relatively short alternations of light and darkness ranging from six hours to five seconds, the plants being illuminated with electric light of high intensity. The plants were grown at approximately outdoor summer temperatures in small light-proof chambers fitted with 1,000-watt Mazda lamps and provided with forced ventilation. In the major series of tests the duration of the light intervals was the same as that of the intervals of darkness in all the alternations. The differential effects of the several alternations on general nutrition and growth were in striking contrast with the comparatively uniform action on initiation of flowering.

As the equal intervals of light and darkness, beginning with 6 hours, were progressively shortened, there was increasing evidence of malnutrition and retardation in growth, which in several instances culminated in the 1-minute intervals. With further shortening of the alternations below the point at which maximum injurious action occurred, there was pronounced improvement in the nutrition and growth of the plants, so that the 5-second alternations often gave about as good results as the 12-hour controls. The injurious effects of the intermediate alternations included apparent destruction of chlorophyll, general etiolation, localized dying of the leaf tissue, reduction in leaf development, attenuation, and decrease in stem elongation and in production of dry matter. The effects on short-day plants and long-day plants seemed to be much the same.

When, with the same total cycles of light and darkness, the light intervals were reduced to one-half the intervals of darkness, the unfavorable effects of the intermediate alternations were intensified to such an extent that most of the plants soon died. On the other hand, increase of the light interval to twice the interval of darkness in each of the cycles had the effect of improving the general appearance of the plants exposed to the intermediate alternations, and in some cases seemed largely to overcome the retarding action of these alternations on growth.

As regards action on flowering, the effects in any particular case seemed to depend mainly on whether the plant belonged to the long-day or short-day type rather than on the duration of the alternations. In general, all the alternations, from 6 hours downward, were found to favor flowering in long-day plants but were unfavorable for flowering in short-day plants. The effects on flowering, therefore, are much the same as those resulting from midday darkening with natural illumination. The plants behave essentially as if exposed to a long day or continuous illumination.

GERMICIDAL EFFICIENCY OF ORTHOPHENYLPHENOL AGAINST MYCOBACTERIUM TUBERCULOSIS[1]

By F. W. TILLEY, *Senior Bacteriologist*, A. D. MACDONALD, *Assistant Veterinarian*, and J. M. SCHAFFER, *Chemist, Biochemic Division, Bureau of Animal Industry, United States Department of Agriculture*

INTRODUCTION

Of the commonly used disinfectants, saponified cresol solution, which consists essentially of phenols dissolved in soap solution, is the only one which has been found to be satisfactorily effective against *Mycobacterium tuberculosis*. This product is readily available on the market and is low in cost. Unfortunately, however, it has a strong odor which is likely to be absorbed by food products exposed to it. This fact limits its usefulness in the work of the Bureau of Animal Industry, practically prevents its employment in packing houses, and gives rise to complaints of tainted milk when it is employed in dairy barns and similar places. For some time past, therefore, a search has been made in the laboratories of the Biochemic Division for a disinfectant that would be efficient but free from objectionable odor. Among the substances studied was orthophenylphenol, one of the higher phenols which is practically odorless when pure. Like many of the higher phenols it is only slightly soluble in water but is readily soluble in the presence of certain soaps, thus affording solutions somewhat similar to saponified cresol solution. It may also be dissolved by the aid of alkalies.

The work, the results of which are reported in this paper, was carried out to determine the germicidal efficiency against *Mycobacterium tuberculosis*[2] of solutions of orthophenylphenol prepared by the aid of soap or alkalies.

EXPERIMENTAL PROCEDURE AND RESULTS

The test organism used in all these experiments was a strain of *Mycobacterium tuberculosis* of the bovine type. In the doses employed in these experiments it regularly caused the death of control guinea pigs within about a month after subcutaneous inoculation. In all experiments the test material, containing this test organism with organic matter in the form of spleen tissue or skim milk, was mixed with the disinfectant, and at the conclusion of the period of exposure portions of each test mixture were inoculated subcutaneously into guinea pigs weighing from 400 to 500 gm. Since orthophenylphenol is practically insoluble in water it was dissolved by the aid of coconut-oil soap or by the use of sodium hydroxide or calcium hydroxide. The amounts of sodium hydroxide or calcium hydroxide were such as to yield the neutral sodium or calcium salt of orthophenylphenol.

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[1] Received for publication Jan. 28, 1931; issued May, 1931.

[2] The bacteriological nomenclature used in this paper is that of the following publication: BERGEY, D. H. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY; A KEY FOR THE IDENTIFICATION OF ORGANISMS OF THE CLASS SCHIZOMYCETES . . . Ed. 3, 589 p., Baltimore. 1930.

In experiment 1 (Table 1) the test material was a suspension prepared by grinding in normal saline solution the spleen of a guinea pig infected by the previously mentioned strain of *Mycobacterium tuberculosis*. Autopsy showed extensive generalized tuberculosis and microscopic examination of the spleen suspension showed the presence of many tubercle bacilli. After the suspension was prepared it was allowed to stand long enough for large particles to settle out. At the time it was used it had a density approximately equal to that of a vigorous bouillon culture of *Eberthella typhi*. Portions of the spleen suspension were mixed with equal volumes of disinfectant solutions of different concentrations and after two minutes' exposure 1 c. c. portions of the various test mixtures were inoculated subcutaneously into guinea pigs. Control animals received ½ c. c. of spleen suspension.

In all other experiments (Table 1) the test material was a suspension of a culture of *Mycobacterium tuberculosis*, grown on glycerinated beef infusion broth, in $\frac{M}{15}$ Na_2HPO_4. At the time it was used this suspension had a density approximately equal to that of a vigorous bouillon culture of *Eberthella typhi*. The test mixtures contained 50 per cent of sterile skim milk, 25 per cent of culture suspension, and 25 per cent of disinfectant solution of different concentrations. At the end of the periods of exposure shown in the table, 2-c. c. portions of each test mixture were inoculated subcutaneously into guinea pigs. Control animals received ½ c. c. of culture suspension.

TABLE 1.—*Germicidal efficiency of orthophenylphenol against Mycobacterium tuberculosis as determined by subcutaneous inoculation of guinea pigs* [a]

EXPERIMENT 1 [b]

Guinea pig No.	Concentrations in test mixtures			Time of exposure	Results of guinea-pig inoculations	
	Orthophenylphenol	Coconut-oil soap	Organic matter and test organism		Termination of test [c]	Lesions of tuberculosis found
				Minutes		
1	1-200	1-100	50 per cent spleen suspension containing test organism.	2	Killed after 72 days	None.
2	1-200	1-100		2	do	Do.
3	1-400	1-200		2	do	Generalized.
4	1-400	1-200		2	do	None.
5	1-800	1-400		2	Died of injury after 18 days.	Slight.
6	1-800	1-400		2	Died after 65 days	Generalized.
7	1-200	1-20		2	Died after 60 days	Do.
8	1-200	1-20		2	Died after 35 days	Do.

EXPERIMENT 2 [d]

9	1-200	1-100	50 per cent skim milk; 25 per cent culture suspension.	2	Killed after 142 days	None.
10	1-200	1-100		2	do	Do.
11	1-400	1-200		2	Died after 54 days	Generalized.
12	1-400	1-200		2	Died after 106 days	Do.

[a] Experiments conducted at room temperature; weight of guinea pigs 400 to 500 gms.
[b] 1 c. c. of test mixture inoculated into test guinea pigs; ½ c. c. into controls.
[c] Life of controls was approximately 30 days.
[d] 2 c. c. of test mixture inoculated into test guinea pigs; ½ c. c. into controls.

TABLE 1.—*Germicidal efficiency of orthophenylphenol against Mycobacterium tuberculosis as determined by subcutaneous inoculation of guinea pigs*—Contd.

EXPERIMENT 3 [d]

Guinea pig No.	Concentrations in test mixtures: Orthophenylphenol	Concentrations in test mixtures: Coconut-oil soap	Concentrations in test mixtures: Organic matter and test organism	Time of exposure	Results of guinea-pig inoculations: Termination of test	Results of guinea-pig inoculations: Lesions of tuberculosis found
				Minutes		
13	1-200	1-100	50 per cent skim milk; 25 per cent culture suspension.	2	Killed after 182 days	None.
14	1-200	1-100		2	Died of intercurrent disease after 102 days.	Do.
15	1-400	1-200		2	Died after 57 days	Generalized.
16	1-400	1-200		2	Died after 56 days	Do.

EXPERIMENT 4 [d]

17	1-200	1-100	50 per cent skim milk; 25 per cent culture suspension.	2	Died of intercurrent disease after 89 days.	None.
18	1-200	1-100		2	Killed after 124 days	Do.

EXPERIMENT 5 [d]

19	1-600	1-300	50 per cent skim milk; 25 per cent culture suspension.	30	Killed after 61 days	Generalized.
20	1-600	1-300		30	do	Do.
21	1-600	1-300		60	Died after 35 days	Do.
22	1-600	1-300		60	Died after 56 days	Do.

EXPERIMENT 6 [d]

23	1-400	1-200	50 per cent skim milk; 25 per cent culture suspension.	15	Died of intercurrent disease after 46 days.	None.
24	1-400	1-200		15	Killed after 124 days	Do.
25	1-400	1-200		30	do	Do.
26	1-400	1-200		30	do	Do.
27	1-400	1-200		60	do	Do.
28	1-400	1-200		60	Died of intercurrent disease after 70 days.	Do.
29	1-600	1-300		60	Killed after 124 days	Do.
30	1-600	1-300		60	do	Do.

EXPERIMENT 7 [d]

31	1-400	(e)	50 per cent skim milk; 25 per cent culture suspension.	30	Killed after 94 days	None.
32	1-400	(e)		30	Died of intercurrent disease after 13 days.	Do.
33	1-400	(f)		30	Died of intercurrent disease after 52 days.	Do.
34	1-400	(f)		30	Killed after 94 days	Do.

[d] 2 c. c. of test mixture inoculated into test guinea pigs; ½ c. c. into controls.
[e] NaOH substituted in quantities sufficient to yield neutral sodium salt.
[f] $Ca(OH)_2$ substituted in quantities sufficient to yield neutral calcium salt.

DISCUSSION

The results given in Table 1 show that after two minutes' exposure orthophenylphenol in a final concentration of 1–200 with a soap concentration of 1–100 was uniformly effective in mixtures containing tubercle bacilli and in the presence of a high percentage of organic matter. The bacilli, as previously noted, were present in large numbers. With the same technic, lower concentrations of orthophenylphenol or the same concentration with a much higher concentration

of soap were not effective. If an allowance of 50 per cent is made for dilution of the disinfectant under practical disinfecting conditions, the foregoing results, together with those reported in a previous paper,[3] suggest that a solution containing 1 per cent of orthophenylphenol and 2 per cent of coconut-oil soap should be effective where rapid action is desired as, for example, in disinfecting the hands.

Results given in the table suggest that for general disinfecting purposes against *Mycobacterium tuberculosis*, where longer exposure is possible, orthophenylphenol in a final concentration of 1–200, dissolved by the aid of coconut-oil soap, sodium hydroxide, or calcium hydroxide, should be effective. Other work, as yet unpublished, indicates that an excess of sodium hydroxide or calcium hydroxide tends to diminish germicidal activity. It is advisable, therefore, to use only as much of these alkalies as may be necessary to dissolve the orthophenylphenol.

SUMMARY

Tests were made with solutions of orthophenylphenol prepared by the aid of coconut-oil soap, sodium hydroxide, or calcium hydroxide, against *Mycobacterium tuberculosis*. After two minutes' exposure orthophenylphenol in a concentration of 1–200 was effective with a soap concentration of 1–100 but was not effective with a soap concentration of 1–20, in the presence of a considerable amount of organic matter. Under similar conditions a concentration of 1–400 with a soap concentration of 1–200 was not effective. Solutions containing orthophenylphenol in a concentration of 1–400 with coconut-oil soap in a concentration of 1–200 or with just enough sodium hydroxide or calcium hydroxide to yield the neutral sodium or calcium salt of orthophenylphenol were uniformly effective after 30 minutes' exposure. Solutions containing orthophenylphenol in a concentration of 1–600 and coconut-oil soap in a concentration of 1–300 were sometimes effective (experiment 7) and sometimes ineffective (experiment 5) after 60 minutes' exposure.

[3] SCHAFFER, J. M., and TILLEY, F. W. GERMICIDAL EFFICIENCY OF SOAP AND OF MIXTURES OF SOAPS WITH SODIUM HYDROXIDE OR WITH PHENOLS. Jour. Agr. Research 41: 737–747. 1930.

THE EFFECT OF SALT ON THE MICROBIAL HEATING OF ALFALFA HAY[1] [2]

By L. S. STUART, *Associate Bacteriologist*, and LAWRENCE H. JAMES, *Senior Bacteriologist, Bureau of Chemistry and Soils, United States Department of Agriculture*

INTRODUCTION

During a study of the heating of moistened hay in the flooded regions of Vermont in 1927 (*6*),[3] reports were received from many farmers who had had marked success in preventing the molding and spontaneous heating of stored hay with a high moisture content by the application of large quantities of salt. No information was available at that time, however, as to the actual rôle of the salt in preventing heating, or as to the quantities which could be used safely without causing injury to livestock. Laboratory studies were undertaken, therefore, to determine the reason for the effect of salt on the microbial heating of organic material.

James, Rettger, and Thom (*7*), who studied heat production in moist organic materials by microorganisms, found that the preliminary heat produced during spontaneous heating was in a large part due to microbial activity. That sodium chloride inhibits the growth of certain microorganisms is well known (*4*). In fact, Duthoit (*1*, *2*, *3*) and others have demonstrated bactericidal action in salt solutions. Shutt (*9*) observed that hay to which salt had been added during the storing process in every case appeared brighter than hay stored unsalted. However, no appreciable chemical differences were noted between salted and unsalted hays. Feeding experiments showed that 20 pounds of salt per ton could be added without bad effects on the animals, but when 30 pounds per ton were added marked physiological effects were noted.

EXPERIMENTAL WORK

James (*5*) devised an apparatus for the study of heat production by microorganisms in moist organic material. This apparatus was adopted for the studies reported here with the exception that air was used as the source of oxygen. The heating flasks were not subjected to forced aeration, but were allowed to draw air freely through the aerating tubes.

To prove that heating which occurred without forced aeration was actually dependent upon oxidation, three flasks were packed with equal quantities of chopped alfalfa hay and rubber policemen fitted to prevent the passage of air through the aerating tubes. To the hay in one (Dewar No. 1) water was added to bring the final moisture content to 40 per cent. To the hay in the other two flasks (Dewar Nos. 2 and 3) salt solutions were added to bring the final moisture content to 40 per cent, and the salt concentrations to 1 and 2 per cent, respectively. The quantity of salt used was determined

[1] Received for publication Dec. 22, 1930; issued May, 1931.
[2] This paper is a joint contribution from the divisions of Chemical Engineering and Food Research, Chemical and Technological Research, Bureau of Chemistry and Soils.
[3] Reference is made by number (italic) to Literature cited, p. 664.

from the total weight of the hay and the water to be added. No significant heating occurred during a period of eight days. (Table 1.)

TABLE 1.—*Heat produced during eight days by moistened[a] chopped alfalfa hay, packed in insulated Dewar flasks, with and without salt, and not aerated*

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 1 per cent salt	Flask No. 3, 2 per cent salt
		°C.	°C.	°C.	°C.
0	12 m.	29.0	28.0	28.0	28.0
	4 p. m.	29.5	27.5	27.5	27.5
1	9 a. m.	29.5	29.0	28.0	29.0
	12 m.	28.0	30.0	29.0	29.0
	4 p. m.	30.0	30.0	29.5	30.0
2	9 a. m.	30.0	32.0	31.0	31.0
	12 m.	31.0	31.5	31.0	31.5
	4 p. m.	30.5	34.5	31.0	31.0
3	9 a. m.	29.5	31.0	30.5	31.0
	12 m.	30.0	31.0	29.0	31.0
	4 p. m.	31.0	31.0	29.5	31.0
8	9 a. m.	31.0	31.0	31.0	31.0

[a] 40 per cent moisture.

These results confirm the observations of other investigators, namely, that heating does not take place in the absence of air or oxygen. The macroscopic appearance of the moistened hay in the Dewar flasks was unchanged except for a slight mold growth on the surface, which penetrated the material about 3 inches.

TABLE 2.—*Heat produced during six days by moistened[a] chopped alfalfa hay packed in insulated Dewar flasks, with and without salt, and aerated*

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 1 per cent salt	Flask No. 3, 2 per cent salt
		°C.	°C.	°C.	°C.
0	10 a. m.	24.5	24.5	24.5	24.5
	4 p. m.	24.5	24.5	24.0	24.0
1	9 a. m.	25.0	26.0	24.5	24.5
	12 m.	25.0	26.0	24.5	24.5
	4 p. m.	27.0	26.0	24.5	24.5
2	9 a. m.	25.5	28.5	24.5	24.0
	12 m.	25.5	28.5	23.5	24.0
	4 p. m.	25.0	30.5	23.5	23.0
3	9 a. m.	25.0	41.0	23.5	23.5
	12 m.	27.0	43.0	25.0	23.5
	4 p. m.	24.0	45.5	25.5	23.0
4	9 a. m.	27.0	47.0	26.5	23.0
	12 m.	26.0	46.5	40.0	23.5
	4 p. m.	26.0	47.5	41.5	25.0
5	9 a. m.	25.0	44.5	44.5	31.0
	12 m.	26.0	44.0	44.0	33.0
	4.30 p. m.	26.0	44.0	44.0	37.0
6	9 a. m.	27.0	44.0	44.5	43.0
	12 m.	26.0	43.5	47.0	45.0
	4.30 p. m.	25.0	44.5	47.0	44.5

[a] 40 per cent moisture.

To confirm the supposition that the flasks would take in enough air for heating without forced aeration, and under such conditions to observe the effect of adding 1 and 2 per cent salt to moist hay, three

flasks were packed with equal quantities of alfalfa hay which had been treated in the same manner as in the preceding experiment. Heating was observed in all flasks, although where 1 per cent salt had been added, heating did not start so soon as in the absence of salt, and where 2 per cent salt had been added heating was further delayed. At the end of six days the maximum temperatures in the three flasks were about the same. (Table 2.) At the conclusion of the heating tests the material in all three flasks contained large quantities of mold growth.

As James, Rettger, and Thom (7) have shown that the quantity of moisture markedly affects the rate of heat development, a series of experiments was conducted in which flasks were packed with equal quantities of alfalfa hay containing varying percentages of added moisture, without salt, and with 1 and 2 per cent salt, respectively. (Table 3.) With 15 per cent moisture, which was the lowest moisture used, 2 per cent added salt was not sufficient to prevent heating, although it did not start until the ninth day, whereas with no salt heating started on the fourth day. With 20 per cent added moisture and no salt, heating occurred within four days, whereas heating did not appear until the sixth day in the presence of 1 per cent salt, and not until the ninth day where 2 per cent salt had been added.

TABLE 3.—*Effect of varying moisture content on the rate of heat development in moistened alfalfa hay, with and without the addition of salt* [a]

Added moisture (per cent)	Heat development in the presence of—					
	No salt		1 per cent salt		2 per cent salt	
	Time	Maximum temperature	Time	Maximum temperature	Time	Maximum temperature
	Days	*° C.*	*Days*	*° C.*	*Days*	*° C.*
15	4	50.0	9	48.5	9	45.0
20	4	43.5	6	44.0	9	43.0
25	2	45.0	5	44.0	8	42.5
30	2	48.0	4	48.0	6	44.5
35	2	41.0	3	41.0	4	41.0
40	2	47.5	3	44.0	5	44.5

[a] Data for this table were compiled in a manner similar to that reported in Table 2.

With 25 per cent or more added moisture and no salt the material invariably started to heat within two days. When 1 per cent salt was added heating started on the fifth day with 25 per cent moisture, on the fourth day with 30 per cent moisture, and on the third day with 35 and 40 per cent moisture. With 2 per cent added salt heating began on the eighth day with 25 per cent moisture, on the sixth day with 30 per cent, on the fourth day with 35 per cent, and on the beginning of the fifth day with 40 per cent.

The maximum temperatures seemed not to be materially affected by the addition of salt. Heat development was more rapid with 30 per cent moisture than with lower percentages of moisture and no addition of salt. This finding substantiates the results obtained in previous investigations.

The results of these experiments indicate that the action of 1 and 2 per cent salt is affected by the quantity of moisture present, namely, with equal quantities of salt, the greater the percentage of moisture the more quickly heating takes place.

The possibility that dry salt may have a greater efficacy in delaying or preventing heat production than the addition of salt in solution was investigated. Alfalfa hay was moistened to a moisture content of 40 per cent. Three flasks were packed with equal quantities. One per cent of salt was added to flask No. 2 and 2 per cent of salt to flask No. 3. The dry salt was sprinkled evenly over the material. The results of this test are given in Table 4.

TABLE 4.—*Heat produced during five days by moistened [a] chopped alfalfa hay, packed in insulated Dewar flasks, with 1 and 2 per cent salt added dry*

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 1 per cent salt	Flask No. 3, 2 per cent salt
		° C.	° C.	° C.	° C.
0	12.00 m.	31.5	31.5	31.5	31.5
	4.30 p. m.	32.0	32.0	31.5	31.5
1	9.00 a. m.	31.5	31.5	31.5	31.5
	12.00 m.	32.0	31.5	31.5	31.5
	4.30 p. m.	32.0	31.5	31.5	31.0
2	9.00 a. m.	29.0	32.5	32.0	32.0
	12.00 m.	29.0	32.5	32.0	32.0
	4.30 p. m.	31.0	33.0	32.0	31.5
3	9.00 a. m.	30.5	45.0	36.0	34.0
	12.00 m.	31.0	45.0	37.0	34.0
	4.30 p. m.	31.0	45.0	38.0	34.0
4	9.00 a. m.	29.5	45.0	47.5	39.0
	12.00 m.	30.0	44.5	47.0	40.0
	4.30 p. m.	30.5	45.0	46.0	41.0
5	9.00 a. m.	27.0	46.0	46.5	43.0

[a] 40 per cent moisture.

No difference could be observed between the action of salt when added in a dry form and in solution. Miehe (*8, p. 116–122*) attributed the greater heating propensity of dung piles that were treated with kainite and mixtures of superphosphate and gypsum in part to the desiccation which resulted from the absorption of water by the salts. The addition of dry salt to large piles of moist or partly moist organic material might have considerable effect upon heating due to the desiccation and possible distribution of moisture throughout the mass. The laboratory heating tests reported in this paper were not large enough to demonstrate such possible physical activities.

Microbial analyses were made of heating alfalfa hay containing 1 and 2 per cent salt, respectively. Bacterial counts were made on the original and test materials on plain agar, plain agar plus 5 per cent salt, and wort agar. (Tables 5 and 6.) In the heating flasks to which no salt had been added large increases of aerobic spore-forming bacteria and molds were noted on plain agar. In many of the flasks to which 1 per cent salt had been added an increase in the numbers of the aerobic spore-forming bacteria occurred, but mold growth seemed to predominate.

TABLE 5.—*Bacterial plate counts from chopped and moistened alfalfa hay, salted and unsalted, that had developed heat*

Salt added to hay	Maximum temperature of heating hay	Incubation period	Total counts on plain agar at 37.5° C.	Total counts on 5 per cent salt agar at 37.5° C.	Total counts on wort agar at 30° C.
Per cent	*° C.*	*Days*			
0	27.5	0	[a] 340,000	[b] 1,440,000	[c] 560
0	45.0	3	[a] 520,000	[b] 580,000	[d]
0	45.0	4	[a] 100,000	[b] 568,000	[d]
0	46.0	6	[a] 1,000,000	[b] 8,000,000	[d]
1	36.0	3	[a] 540,000	[b] 340,000	[d]
1	47.5	4	[a] 6,480,000	[b] 7,280,000	[d]
1	46.5	6	[d]	[b] 19,680,000	[d]
2	34.0	3	[a] 32,400	[b] 242,000	[d]
2	39.0	4	[a] 120,000	[b] 146,000	[d]
2	43.0	6	[a] 140,000	[b] 30,560,000	[b] 7,200,000

[a] Subtilislike colonies.
[b] Pin-point colonies only.
[c] Species of Aspergilli and Penicillia.
[d] Plates covered with species of Aspergilli and Penicillia.
[e] Plates covered 1:1,000,000 dilution.

In the flasks to which 2 per cent salt had been added the results were somewhat different. In every case the plain agar counts from this material were less than those on the original unpacked hay. This was true even after the flasks had been held for as long as 9 to 10 days. Salt agar plates from the original material gave high counts of pin-point colonies, which proved on examination to be largely gram-positive cocci and bacilli. Increases in the counts on salt agar did not occur until after heating had been continued over a prolonged period. It would seem, therefore, that such increases are not associated with the actual rise in temperature, but rather are a result of the incubation effect produced by the growth of other organisms. However, it is possible that the action of these microorganisms assisted in maintaining and prolonging high temperatures. Wort agar plates showed marked increases in the number of mold colonies as heating progressed.

The delay in heating in the flask that contained 1 per cent salt as compared with the flask to which no salt was added can not definitely be accounted for, although the results of the experiments would seem to show that it was in part owing to the inhibitive effect of salt on certain bacteria and in part to an increase in the "lag" period of the salt-tolerant microorganisms present.

The increased delay in the flasks containing 2 per cent salt could then be attributed to an increased efficiency of the same action. In fact, results would seem to indicate that 2 per cent salt entirely eliminates the factor of heating from bacterial action, leaving only the heating from the mold growth.

The data in Table 6 show a marked difference in the microbial counts during heating, suggesting variations in the types of organisms present. In order to obtain more definite information relative to the effect of salt in modifying the microbial flora, four flasks were packed with moistened chopped alfalfa hay, and salt was added to two flasks, 1 and 2 per cent, respectively. Examinations were made in the first before heating began, and in the other three when a temperature of 41° C. had been reached. The results given in Table 6 show that anaerobes are present in fair quantities throughout; that lactose ferments and gelatin liquefiers and other organisms which grew at 20° are inhibited by salt; that plain agar and 5 per cent salt agar counts are similar to those given in Table 5; and that yeasts and molds markedly increase in all cases.

TABLE 6.—*Microbial analyses of heating chopped moistened alfalfa hay with and without the addition of salt* [a]

Salt added to hay	Incubation period	Maximum temperature of heating hay	Total counts when cultured in—							
			Lactose broth at 37.5° C.	Anaerobic beef broth at 37.5° C.	Gelatin at 20° C.	Plain agar at 37.5° C.	Plain agar at 37.5° C. (Spore count [b])	5 per cent salt agar at 37.5° C.	Dextrose agar at 37.5° C.	Wort agar at 30° C.
Per cent	*Days*	*° C.*								
0	0	26.5	Gas in 0.1 dilution	Putrefactive odor in 0.1 dilution.	202,000. Liquefiers, 1,420.	[c] 840,000	[c] 152,000	[d] 16,400	[c] 302,000	[e] 38,000
0	3½	41.0	do	do	30,000. Liquefiers, 10,000.	[c] 17,000,000	[c] 23,600,000	[d] 480,000	[e] 20,400,000	[e] 4,400,000
1	4	41.0	do	do	No growth in 48 hours	[c] 2,400,000	[d] 280,000	[d] 180,000	[c] 320,000	[e] 3,800,000
2	6	41.0	No gas in 0.1 dilution	do	do	[d] 64,000	[d] 44,000	Mold growth only.	28,000 (Few molds)	[e] More than 10,000,000.

[a] Counts made on water washings of 10-gram samples recorded as counts per gram.
[b] Spore counts made by heating sample to 80° C. for 20 minutes, immersing quickly in cold water and plating.
[c] Subtilis and mycoideselike colonies.
[d] Pin-point colonies only.
[e] Species of Aspergilli, Penicillia, and Oidia.

All flasks that had heated contained large quantities of mold growth. An experiment was now conducted to determine, if possible, the quantity of salt necessary to prevent molding and subsequent heating. Three flasks were packed, containing no salt, 4 per cent salt, and 5 per cent salt, respectively. The results of this experiment showed that 5 per cent salt per total weight of hay (containing 30 per cent moisture) was not sufficient to prevent molding and heating. (Table 7.) The flasks showed a heavy mold growth.

TABLE 7.—*Heat produced during 13 days by moistened [a] chopped alfalfa hay, packed in insulated Dewar flasks, with 4 and 5 per cent salt*

Days packed	Hour observed	Temperature in—			
		Room	Flask No. 1, no salt	Flask No. 2, 4 per cent salt	Flask No. 3, 5 per cent salt
		°C.	°C.	°C.	°C.
1	10.00 a. m.	27.0	27.0	27.0	27.0
2	9.00 a. m.	27.5	27.5	27.0	27.0
	4.00 p. m.	27.0	27.5	26.5	27.0
3	9.00 a. m.	25.0	26.5	26.0	26.0
	12.00 m.	26.0	26.5	26.0	26.0
4	9.00 a. m.	24.0	27.0	26.0	26.0
7	9.00 a. m.	25.0	45.5	27.0	27.0
	12.00 m.	26.0	45.0	27.0	27.0
	4.00 p. m.	27.0	44.5	27.0	27.0
8	12.00 m.	32.0	46.0	30.0	30.0
	4.00 p. m.	29.5	46.0	29.5	29.5
9	9.00 a. m.	26.5	46.0	29.5	29.5
	12.00 m.	26.0	46.0	29.5	29.5
	4.00 p. m.	28.5	45.5	29.5	29.5
10	9.00 a. m.	30.5	46.0	30.0	30.5
	12.00 m.	29.0	47.5	32.5	30.5
	4.30 p. m.	29.0	47.5	32.5	30.5
11	9.00 a. m.	29.5	49.0	39.0	34.0
	12.00 m.	29.0	48.5	41.0	34.5
12	9.00 a. m.	29.0	48.5	43.5	42.5
13	9.00 a. m.	28.5	47.0	46.0	44.5

[a] 30 per cent moisture.

Since even 5 per cent salt would be far in excess of the 30 pounds per ton found to have a marked physiological effect upon cattle (*9*), it would seem that the quantity of salt necessary to prevent molding would be far in excess of the quantity that could safely be added to hays under actual practical conditions. However, addition of salt to slightly damp and spotted hays might delay heating until the moisture content had lowered, through evaporation or equalization, below the critical point for mold growth. This could be demonstrated effectively only in large-scale experiments.

SUMMARY

Moistened alfalfa hay packed in a Dewar flask into which an aerating tube had been inserted and left open heated to 47.5° C. When the aerating tubes were closed, no heating was observed.

Moistened alfalfa hay to which 1 per cent salt had been added did not start to heat so soon as hay to which no salt had been added, and when 2 per cent salt was added heating did not start so soon as when 1 per cent salt been added.

Flasks filled with alfalfa hay with different moisture contents, with no salt and with 1 and 2 per cent salt, showed that with 30 per cent moisture and no salt, heat development was more rapid than with

lower percentages of moisture, and that the delay in heating when salt was added varied inversely with the quantity of moisture present.

No difference was noted between the action of dry salt and salt in solution.

Bacteriological examination (1) of unsalted hay at various stages of heating showed marked increases in mold and aerobic spore-forming bacteria as the heating progressed, (2) of hay to which 1 per cent salt had been added showed marked increases in mold growth as heating progressed, and (3) of hay to which 2 per cent salt had been added showed marked decreases in bacterial counts and increases in mold growth as heating progressed.

Alfalfa hay containing 5 per cent salt and 30 per cent moisture molded and heated within 12 days.

Salt added to moistened alfalfa hay will inhibit bacterial growth and will delay but not prevent mold development. This delay in microbial development may be long enough to permit the curing of the hay.

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A PRELIMINARY STUDY OF THE DETERMINATION OF THE APPARENT DIGESTIBILITY OF PROTEIN BY MODIFIED PROCEDURES [1]

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INTRODUCTION

Modifications of the usual methods employed in the determination of the apparent digestibility of food are of practical importance in so far as they yield more accurate results or simplify technical details relative to such determinations. A recent modification has been suggested by Bergeim (*1*),[2] who recommends the addition to the food of iron oxide or other suitable substance which is excreted with practical completeness in the feces. The digestibility coefficients are then calculated from a determination of the ratio of food substance to iron in the diet and as found in the feces. This method offers simplicity, since a measure of the amount of food consumed and feces excreted during an experimental period is not necessary. Its precision as a method for determining the composition of feces from which digestibility data are obtained appears to be limited to the accuracy of the iron determinations and the maintenance of a constant ratio between iron and food constituents in the diet.

In a previous study of the digestibility of protein, in which this method was employed, one of the authors (*4*) obtained results comparable to those obtained by the usual method, in which the total amount of food eaten and that excreted was determined and the digestibility calculated by difference. Heller, Breedlove, and Likely (*7*) believed that greater accuracy could be attained with Bergeim's method if the normal iron content of the diet were used as a reference substance, since practically all of the iron appears in the feces and any errors produced through an uneven distribution of the iron oxide in the food are eliminated. Other experiments (*5*) have demonstrated the practicability of substituting silica for iron oxide under conditions in which the iron can not be recovered quantitatively from the feces.

All of the foregoing experiments have been conducted with rats as experimental animals and under conditions in which a measure of the food eaten and the daily collection of feces have not presented great difficulty. Bergeim (*1, p. 32*), in commenting on his proposed method, states: "The extent to which this procedure may replace the usual method can only be demonstrated by experiments carried out under a variety of conditions." The results obtained in the following experiment indicate the extent to which modified procedures may be employed in digestibility studies with large animals where all the difficulties of the older method of experimentation pre-

[1] Received for publication Jan. 19, 1931; issued May, 1931.
[2] Reference is made by number (italic) to Literature Cited, p. 669.

vail. The limited value of digestibility coefficients obtained in this way has been fully discussed by Mitchell (*8*).

EXPERIMENTAL DATA

The usual precautions necessary in conducting a digestibility study were observed throughout the experiment. The experimental animals were four Jersey heifers approximately 1½ years old and weighing about 500 pounds. The rations were similar to those being used in nutrition studies with cottonseed meal. Their composition is presented in Table 1 in such a manner as to show the daily food allowance.

TABLE 1.—*Composition of rations used in nutrition studies with cottonseed meal*

[NaCl was fed ad libitum]

Constituent	Quantity in ration No.[a]—			
	21	22	25	26
Cottonseed meal.....pounds	5.5	5.5		
Cottonseed meal (autoclaved).....do			5.5	5.5
Prairie hay (chopped).....do	8.0	8.0	8.0	8.0
$CaCO_3$.....grams		15.0		15.0
Fe_2O_3[b].....do	13.0	13.0	13.0	13.0

[a] This number also refers to the laboratory number of the animal. In reality only two rations were made up since the $CaCO_3$ was added at the time of feeding.
[b] Approximate values are given for Fe_2O_3.

Choice cottonseed meal containing 43 per cent crude protein was used in these experiments. The autoclaved meal was prepared in the laboratory by cooking the meal under 25 pounds of steam pressure for 30 minutes. This process, which is effective in destroying much of the gossypol that may be present in the commercial meal, has been discussed elsewhere (*4*).

The feeding trial lasted 10 days, during which time four samples of each ration were taken for proximate analysis and iron determinations. The results of the analyses are presented in Table 2. Samples of fresh feces were collected in the morning and evening during five days of the experiment. Composite samples of the last three daily collections were used for the calculation of the coefficients of digestibility. The results are presented in Table 3, in which periods 1, 2, and 3 represent the last three days of the experiment. Iron determinations were made by following a colorimetric method outlined by Bergeim (*2*), special care being exercised in dissolving the iron and maintaining a constant acidity in all of the solutions. The results, which are presented in Table 3, are the average values obtained from four or more analyses. The difficulties encountered in using this method for the determination of fecal iron were of the same nature as have been previously reported (*4*, *7*), and are accounted for as being due to interfering substances which were present in relatively greater amounts in the feces than in the feed.

To secure what appears to be more reliable data, the silica content of the feed and feces was determined and the digestibility coefficients calculated from the ratios so obtained. Greenwald and Gross (*6*) have used a similar method for studying the utilization of calcium

and phosphorus. It has been reported (*3*) that Wiedt fed sheep wheat straw and used the silica naturally contained therein as an index of the absorption of other substances.

TABLE 2.—*Iron determinations and proximate analyses of rations expressed in percentage*

Ration Nos.	Iron found in ration sample No.—					Proximate analysis of rations				Silica
	1	2	3	4	Average	Protein	Nitrogen-free extract	Fat	Fiber	Average
21-22	0.137	0.145	0.148	0.145	0.144	18.40	42.50	4.10	22.10	3.21
25-26	.115	.118	.116	.114	.116	18.23	41.94	3.54	22.83	3.23

TABLE 3.—*Protein, iron, and silica: Feces and results obtained by two different methods of calculating the apparent digestibility of the protein contained in similar rations*

Period No.	Protein in feces	Iron in feces	Silica in feces	Digestibility coefficient by—	
				Iron method	Silica method
RATION 21					
	Per cent	*Per cent*	*Per cent*		
1	11.84	0.320	7.24	71.1	71.5
2	11.64	.273	6.81	66.7	70.2
3	11.45	.318	6.67	71.8	70.1
RATION 22					
1	12.56	0.364	8.17	73.0	73.2
2	11.78	.336	7.73	72.3	73.5
3	11.78	.260	6.88	64.3	70.1
RATION 25					
1	15.15	0.316	7.95	69.5	66.3
2	16.44	.331	9.50	68.4	69.9
3	13.23	.295	7.20	71.5	67.5
RATION 26					
1	14.40	0.350	7.48	73.8	66.6
2	13.37	.250	6.41	65.6	63.1
3	13.93	.288	8.18	68.6	69.8

DISCUSSION

An even distribution of iron oxide through the ration is of primary importance, and the figures presented in Table 2 are taken as attesting to the completeness with which such a distribution may be made. There were no uneaten food residues, and since an examination of the containers after feeding did not show a separation of the added iron, it was assumed that the animals were eating a ration in which

the ratio of iron to other food constituents was definite and did not vary from day to day. Furthermore, analyses of feces obtained at intervals during the day showed only slight differences in composition which were in accord with the small daily variations to be expected in experiments of this kind. It appears highly probable, therefore, that variations in the digestibility coefficients which were calculated from the iron ratios and which are outside the natural limits of experimental error are caused by inaccuracies in the iron determinations. This possibility is suggested, although Bergeim (*1*) has stated that if 10 per cent of a given food substance is not digested a 10 per cent error in the analyses will produce only a 1 per cent change in the digestibility value. The amount of undigested substance is oftentimes much greater than this, and as a consequence small errors in the iron determinations produce conspicuous variations in the digestibility coefficients.

The lack of reliable methods for the rapid determination of iron under a variety of conditions, some of which predominate in digestibility studies, has been brought out by Heller (*7*) and discussed by many workers in papers dealing with iron metabolism. As a result, elaborate methods have been suggested which are highly accurate but are time consuming and for the most part do not qualify where simple technic is desired. Perhaps no one method will serve under all conditions, which, if true, precludes the possibility of outlining in detail a standard procedure for conducting digestibility studies in this manner. An investigation of other substances to be used in place of iron oxide under a variety of conditions would seem worth while.

The determination of the silica content of the feed and feces presented no difficulties, and the digestibility coefficients so calculated were in close agreement and of the same order as those calculated from the iron ratios, Because of the ease with which these determinations were made, this method gave promise of being preferable to the iron method. The coefficients so obtained, and presented in Table 4, agree fairly well with those which have been reported for the proteins in the rations used. Watkins (*9*) has recently reported the crude protein of a ration made up of cottonseed meal and straw with a nutritive ratio of 1:2.8 to be 75.7 per cent digestible. The nutritive ratio in the rations used by the writers was 1:2.4.

TABLE 4.—*Average digestibility coefficients of various rations calculated from the silica content of the feed and feces*

Ration No.	Digestibility coefficient of—			
	Protein	Fat	Nitrogen-free extract	Fiber
21	70.6	66.9	53.2	50.2
22	72.2	71.9	57.9	52.3
25	67.9	69.4	62.0	59.4
26	66.5	66.3	57.7	51.9

In an experiment with rats (*4*) that were receiving a diet containing 18 per cent protein derived solely from cottonseed meal, the apparent digestibility of the protein was found to be 71.5 per cent. In the experiments with dairy heifers reported here, the decrease in the digestibility of the protein produced by autoclaving the meal was not great, although in experiments with rats the digestibility was decreased nearly 10 per cent by this process. The presence of $CaCO_3$ in the rations had no appreciable effect.

SUMMARY

A digestibility trial with large animals has been successfully carried out by modified procedures. The apparent digestibility of the protein was calculated from a determination of the ratio of the amount of protein to both iron and silica in the feed and in the feces. An average value of 71.9, which agrees closely with that reported in earlier work for the digestibility of cottonseed-meal protein, was obtained.

Under the conditions of the experiment, the silica naturally contained in the feed served as a better index of the digestibility of the other substances than did the iron which had been added for that purpose.

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CHANGES IN THE SUGAR, OIL, AND GOSSYPOL CONTENT OF THE DEVELOPING COTTON BOLL[1]

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INTRODUCTION

Present knowledge of the rate of formation of gossypol in cotton seeds as related to the accumulation of oil, protein, and certain other constituents of the cotton plant has been discussed and summarized in previous publications (*2*, *3*).[3] The present investigation is a continuation of these studies of gossypol, and deals with the changes in sugar, oil, and gossypol which occur at different stages in the development of the cotton boll. The method of procedure used in this investigation offered a means of testing the validity of previous findings on the development of oil in the seeds and afforded an opportunity to observe chemical changes during much shorter intervals of growth than had previously been reported.

EXPERIMENTAL PROCEDURE

One thousand cotton blossoms of the Oklahoma Triumph 44 variety which had been open for less than 2 days were marked by means of tags bearing the approximate date at which the flowers opened. This date was used to determine the age of the bolls that were collected at the end of 21 days and every 3 days thereafter. A 21-day period was selected as the time for the first collection because less mature bolls contain very little ether-soluble material. The last collection was made on the fiftieth day, when all the bolls had reached the mature stage. A description of the material so collected is included in Table 1.

The separation of burs, lint, and seeds was made in the laboratory by hand and the dry weight determined on a small sample of each. One portion of seeds was dried at a sufficiently low temperature (50° C.) to prevent the destruction of gossypol and another portion was preserved in 80 per cent alcohol for sugar determinations. Composite samples of burs and lint were similarly preserved for sugar determinations. The amount of ether-soluble extract in the seeds, which is taken as representing oil, was determined on the dry residue from the moisture determinations. Gossypol determinations were made on 40 grams of the air-dry seeds after the manner outlined by Schwartze and Alsberg (*5*). Reducing sugars were determined by the Shaffer-Hartman method (*6*) on the alcoholic extract of the above material. The extracts, after evaporation to a small volume, were taken up with water several times and finally clarified with neutral lead acetate. Excess lead was removed with potassium oxalate (*4*)

[1] Received for publication Jan. 3, 1931; issued May, 1931.
[2] The authors are indebted to Dr. J. E. Webster for much valuable assistance in making the sugar determinations.
[3] Reference is made by number (italic) to Literature Cited, p. 673.

and the filtered solutions made to definite volume. Aliquots of these were used for determining the reducing and total sugars. The total sugars were determined after hydrolysis with citric acid according to the procedure described by Webster and Dalbom (*7*). The sugar equivalents in terms of dextrose were obtained from Munson and Walker tables (*1*).

EXPERIMENTAL RESULTS

The gossypol, oil, and sugar content of cotton bolls at different stages of maturity is shown in Table 1. An examination of this table discloses the fact that there is a concurrent formation of oil and gossypol in the seeds with a rapid increase of each during 10 days of growth. The relatively more rapid increase of gossypol than of oil during this early period of growth is brought out by calculating the ratio of oil to gossypol which decreases from 159:1 at 21 days to 58:1 at 34 days and does not fall below 50:1 thereafter. The critical period of oil formation appears to be between the twenty-first and thirtieth day. The sugar changes do not lend themselves to interpretation in relation to gossypol but show a more gradual and continued decrease as the seeds mature. Nor do the figures give convincing evidence of the formation of oil at the expense of sugar, except as they show a decrease in sugars as the oil increases.

TABLE 1.—*Gossypol, oil, and sugar content of separate parts of the cotton boll at different stages of maturity*

Age, days	Moisture	Dry weight from 100 bolls	Percentage content of various constituents on a dry-matter basis			
			Oil	Reducing sugar [a]	Total sugar [a]	Gossypol
	Per cent	*Grams*				
SEED						
21	75.2	174	8.26	2.78	4.60	0.052
24	73.5	177	11.73	2.90	3.20	.113
27	70.0	225	16.66	1.97	2.30	.168
30	65.0	246	20.55	1.57	1.86	.266
34	56.8	302	20.25	.81	1.23	.347
37	52.0	316	20.80	.56	1.08	.360
41	26.6	386	[b] 22.30	.16	.42	.356
44	17.7	329	20.20	.12	.32	.399
50	23.2	274	20.90	.15	.32	.408
LINT						
21	70.25	132		11.35	10.98	
24	70.25	137		7.90	8.57	
27	65.8	141		7.60	7.80	
30	53.5	222		3.43	3.55	
34	47.5	241		2.89	3.40	
37	48.5	205		1.94	2.30	
41	17.0	208		.41	.55	
44	12.0	205		.23	.38	
50	12.0	202		.17	.28	
BURS						
21	80.85	164		3.67	3.75	
24	82.61	142		[b] 6.73	[b] 7.00	
27	80.66	144		3.69	4.77	
30	80.50	159		3.90	4.61	
34	78.00	184		2.37	3.05	
37	80.00	138		2.00	2.93	
41	65.00	144		.96	1.05	
44	49.00	154		.58	.69	
50	18.40	154				

[a] Calculations in terms of dextrose.

[b] Values are probably incorrect.

SUMMARY

The sugar, oil, and gossypol content of cotton bolls at different stages of maturity was determined. Both gossypol and oil increased rapidly in the seed from the twenty-first day until the thirtieth day. The gossypol increased more rapidly than the oil, and continued to increase slowly until the boll was 50 days old and fully matured. The sugars in all parts of the boll decreased gradually during the 30-day period of growth.

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THE EFFECT OF PASTEURIZING AND HOMOGENIZING TEMPERATURES ON CERTAIN PROPERTIES OF ICE-CREAM MIXES[1]

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INTRODUCTION

Standard or uniform pasteurization temperatures have never been adopted by the ice-cream industry; consequently the temperatures used vary widely. The same may be said of the temperatures employed for the homogenization of the mix, for in the majority of cases the mixes are homogenized at the pasteurizing temperature. The lack of uniform standards prompted this study.

In the market-milk industry the use of pasteurizing temperatures between 142° and 145° F. for a period of 30 minutes is the rule. Following the example set by the market-milk industry, ice-cream manufacturers until recent years have pasteurized the mix at a temperature of about 145° and held it at this temperature for a period of 30 minutes. This temperature was considered effective for the killing of pathogenic bacteria. Hammer and Sanders (*6*)[2] found considerable destruction of bacteria at temperatures of 142° to 150°. Fabian and Cromley (*4*) found that a temperature of 150° maintained for 30 minutes destroyed 94.5 to 99.9 per cent of the organisms present, and Fay and Olson (*5*) reported similar results.

The ice-cream industry has been satisfied to use a pasteurizing temperature of 145° F. for 30 minutes because of the low cost of fuel as compared to that when higher temperatures are used, and because it was believed that higher temperatures would injure certain qualities of the ice-cream mix. So fearful of excessive heat were some manufacturers, that they practiced cooling the mix from the pasteurizing temperature to 110° or 120° before passing it through the homogenizer or viscolizer, as otherwise some of the mix would be held at the pasteurizing temperature for a period of one to one and one-half hours, depending on the size of the batch and the capacity of the equipment used. The homogenization of the mix at the lower temperatures resulted in greatly increased viscosity unless the homogenizing pressure was reduced. This high viscosity was desired, since the manufacturer believed that the mix would not whip properly unless it had a fairly high viscosity.

Late years have brought changes in the temperatures employed for pasteurizing and homogenizing. Instead of the temperatures being reduced after pasteurizing and before homogenizing, the mix is usually passed through the homogenizer at the pasteurizing temperature. This practice enables the manufacturer to use higher homogenizing pressures without the danger of obtaining an excessive viscosity in the mix.

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[2] Reference is made by number (italic) to Literature Cited, p. 688.

City ordinances and public health officials are calling for greater bacteria-killing efficiency and safety in dairy products. Since higher temperatures do not injure ice-cream mixes in the way that they injure milk, and since ice-cream mixes are composed of numerous products, both dairy and others, it has been found desirable to use higher temperatures in their pasteurization. Some ordinances are known which require temperatures of 145°, 150°, and 160° F. for 30 minutes.

The prejudice against high temperatures has lessened until now we find higher temperatures are advocated for reasons which were not considered formerly. Hening (*7*), using temperatures of 145°, 155°, and 165° F. for 30 minutes and 180° for 10 minutes, found that the higher temperatures decreased the viscosity of mixes and the size of the fat clumps and improved the whipping properties. The mixes that were pasteurized and homogenized at 180° showed the lowest viscosity, the greatest overrun, and the fewest and smallest fat clumps. The flavor of this ice cream had a cooked aftertaste, while the flavor of the mixes pasteurized at lower temperatures did not. Hening stated that—

> The temperature of 180° F. is impractical, but it changes the properties of the mix to the greatest extent. If the temperature of 180° F. was the only one at which these properties of the mix were altered it would show a possibility of the change being due to calcium precipitation.

Martin, Swope, and Knapp (*8*), using temperatures of 145°, 155°, and 165° F., and holding periods ranging from 15 minutes to 2 hours, found that the overrun and the length of time required for freezing were practically the same in all trials and that no temperature or holding period was definitely superior to any other. They concluded that the quality of the finished ice cream was slightly better at "the lower" temperature of pasteurization. They also found that the acidity and hydrogen-ion concentration of the mixes remained practically unchanged after the mixes had aged for 48 and 72 hours at 40° F.

Olson (*9*) stated that, other factors remaining the same, the texture and whipping properites of the mix were much improved by higher temperatures, and the viscosity was reduced. When the temperature went much above 150° F., however, a candy flavor developed.

Dahle, Keith, and McCullough (*2*) used pasteurization temperatures of 150° F. for 30 minutes, 160° for 20 minutes, and 170° for 10 minutes and found that the higher temperatures of pasteurization and homogenization produced mixes lower in viscosity than did the lower temperatures. The mixes pasteurized at the higher temperatures also whipped faster.

SCOPE OF THE INVESTIGATION

There are few data available that show the effect of pasteurization and homogenization temperatures on the physical properties of ice-cream mixes. All effects resulting from high temperatures have usually been attributed to pasteurization temperatures. One of the objects of the present study was to determine the effect of the temperature at which the mix is homogenized on its physical properties, as preliminary work had indicated that the homogenization temperature was responsible for some of the results obtained.

Another object of the study was to devise some means of utilizing high temperatures and at the same time overcoming the apparent disadvantage of holding mixes at the high temperature for long periods during homogenization.

METHODS

Two mixes of different composition were used. One was calculated to test 12 per cent fat, 10.5 per cent serum solids, 15 per cent sugar, 0.36 per cent gelatin, and 37.86 per cent total solids; the other to test 10.5 per cent fat, 12 per cent serum solids, 15 per cent sugar, 0.36 per cent gelatin, and 37.86 per cent total solids.

The mixes were made for the most part from cream, condensed skim milk, skim milk, sugar, and gelatin. In some cases dry skim milk was used, and where a study was made of the effect of pasteurizing and homogenizing temperatures on mixes containing butter, this product was also used. The control mixes always contained cream, condensed skim milk, skim milk, sugar, and gelatin.

Three pasteurizing temperatures were used throughout the experiment, 150° F. for 30 minutes, 170° for 10 minutes, and 180° for 1 minute. Each mix except that pasteurized at 150° was divided into two parts before homogenizing, for the purpose of studying the effect of homogenizing temperatures. One part was homogenized at the pasteurizing temperature and the other part was cooled from 170° or 180° to 150° before homogenizing; in this way the effect of homogenizing temperatures was studied.

Two systems of pasteurizing were used, the vat or holding method and the continuous method. In the holding method the mixes were heated in 10-gallon milk cans. Enough mix was used to provide 5 gallons for each freezing. In the continuous method the ingredients of the mix were added to a 250-gallon glass enameled pasteurizer and heated to 150° F., and the sample to be homogenized at 150° was then removed and held at that temperature for 30 minutes. The mixes heated to higher temperatures were passed through a centrifugal heater which raised the temperature to 170° or 180° as desired just before the mix passed through the homogenizer. The mix remained at these high temperatures only momentarily as it was passed over a surface cooler directly from the homogenizer. One-half of the batches heated to 170° and 180° were drawn off and cooled to 150°, as mentioned before. Table 1 shows the processing temperatures used.

TABLE 1.—*Temperatures used in pasteurizing and homogenizing ice-cream mixes by different processes*

Process No.	Pasteurizing temperature	Time maintained	Homogenizing temperature
	°F.	*Minutes*	°F.
1	150	30	150
2	170	10	170
3	170	10	150
4	180	0	180
5	180	0	150

For studying the freezing conditions, a 40-quart horizontal brine freezer was used. A preliminary batch of ice cream was frozen before the experimental batches were frozen in order to insure a constant freezer temperature for all batches. The brine temperature was kept as nearly constant as possible during the freezing of each series. The brine was shut off the freezer when a definite consistency in the ice cream was obtained. This consistency was determined by means of an indicating ammeter device and by observation. Overrun and temperature readings were made at the end of each minute. A Fahrenheit thermometer graduated to 0.1 degree was used.

Samples of mix were taken immediately after the cooling operation, and laboratory determinations were made on these samples after 4 hours of aging and after 24 hours of aging. Viscosity determinations were made with the MacMichael viscosimeter at 20° C., using a 3 cm. bob and a No. 28 wire calibrated with a standard sucrose solution. Hydrogen-ion determinations were made by means of a Leeds and Northrup type K potentiometer, using the quinhydrone electrode at exactly 25° C. Titratable acidity determinations were made with Mann's acid test, using N/10 sodium hydroxide with phenolphthalein as an indicator.

Protein-stability determinations were made on 5 c. c. samples of mix to which 10 c. c. of a mixture of 95 per cent alcohol and distilled water were added to produce the first positive signs of feathering or coagulating. The number of cubic centimeters of alcohol in the 10 c. c. sample of alcohol and water was used to indicate the stability of the proteins. The greater this number, the greater is the stability of the proteins.

A Du Nuoy tensiometer was used in determining surface tension. The samples of mix were tempered in a constant temperature water bath to 20° C. Three readings were taken on each mix and the average recorded.

To determine the degree of fat clumping, a hanging-drop slide was made of mix diluted 1 to 300. After examination under the low and high powers of the microscope, the degree of clumping was recorded in numbers, as follows: 0 = no clumping, 1 = evident clumping, 2 = very evident clumping, 3 = prominent clumping, 4 = very prominent clumping, 5 = pronounced clumping, 6 = very pronounced clumping.

The results of the work recorded represent averages of at least three separate trials with every mix used. In some cases averages of many more trials are recorded. A total of 145 mixes was prepared, pasteurized, frozen, and tested in various ways. Twenty-six mixes were prepared and pasteurized and homogenized at each temperature and 15 additional control mixes were made.

EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 12 PER CENT FAT MIXES WHEN THE VAT METHOD OF HEATING WAS USED

Eight series of mixes containing 12 per cent fat, 10.5 per cent serum solids, 15 per cent sugar, 0.36 per cent gelatin, and 37.86 per cent total solids were made and pasteurized by the vat or holding method of pasteurization. The source of butterfat was cream in four mixes, and unsalted butter in four mixes. The source of additional serum solids was divided equally between skim-milk powder and condensed skim milk.

The effect of processing temperatures on the viscosity of the mixes and fat clumping is plainly shown in Table 2. There it may be seen that the higher the temperature used the lower was the viscosity and the tendency to clump. When the mixes were homogenized at 170° and 180° F. the viscosity was at a minimum. When the mixes were cooled from these temperatures to 150° before homogenizing, the viscosity was greater, showing the importance of homogenizing at the higher temperatures when the mix is pasteurized at these temperatures. The viscosity and fat clumping were greatest in the mixes that were both pasteurized and homogenized at 150°. The degree of fat clumping followed the viscosity closely. The higher temperatures caused a very marked reduction in the fat clumps, except where the temperature was reduced to 150° for homogenization.

TABLE 2.—*Effect of processing temperatures on physical, chemical, and freezing properties of 12 per cent fat mixes when using the vat method of heating*

[Average of eight trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, ° F.	150	170	170	180	180
Homogenizing temperature, °F.	150	170	150	180	150
Viscosity, centipoises	236.98	91.76	173.42	75.80	173.10
Titratable acidity, c. c. N/10 NaOH	.125	.168	.173	.167	.165
pH	6.33	6.33	6.33	6.35	6.34
Fat clumping [a]	4	.87	2.8	.37	2.37
Surface tension, dynes	46.25	46.35	46.38	46.41	46.36
Protein stability, c. c. alcohol	5.17	5.4	5.45	5.57	5.47
Time to obtain 100 per cent overrun, minutes	8.26	7.40	7.85	7.23	7.62
Total score [b]	72.31	72.16	72.20	72.33	72.27

[a] 0=None, 6=most.
[b] Perfect=80. Average of scoring by 3 judges.

In this series of trials no particular difference was noted in the surface tension or protein stability due to different temperatures of heating.

The lower temperatures of pasteurizing and also of homogenizing caused an increase in the time required to obtain 100 per cent overrun. While it is not always possible to show a relationship between the whipping ability of a mix and viscosity, it is commonly found that the mixes having the highest viscosity, especially when this is due to fat clumping, whip most slowly. This was generally true in this experiment.

If the results at the highest temperatures are compared, it will be seen that there was some difference between those at 170° and 180° F. when the mix was both pasteurized and homogenized at these temperatures. The higher temperature reduced viscosity and fat clumping more than did the lower. From the standpoint of protein stability and surface tension there was very little difference noted in the results at 170° and 180°.

Practically no difference was found in the score of the different batches of ice cream.

EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 12 PER CENT FAT MIXES WHEN THE CONTINUOUS METHOD OF HEATING WAS USED

In this experiment the mix was prepared in a 250-gallon pasteurizer. When the mix had reached a temperature of 150° F. it was run through the continuous pasteurizer at the desired temperature and discharged

into a small tank before it passed through the homogenizer. The mix that was cooled from 170° and 180° to 150° before homogenizing was removed from this tank and cooled in 10-gallon milk cans to 150° and then passed through the homogenizer.

Five trials with mixes testing 12 per cent fat and 37.86 per cent total solids were made. The average of these trials appears in Table 3. Three of the mixes contained butter and two contained cream as a source of butterfat. Condensed skim milk furnished the extra serum solids in all mixes except one, in which powdered skim milk was used.

TABLE 3.—*Effect of processing temperatures on physical, chemical, and freezing properties of 12 per cent fat mixes when using continuous method of heating*

[Average of five trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, ° F.	150	170	170	180	180
Homogenizing temperature, °F.	150	170	150	180	150
Viscosity, centipoises	2,282.4	98.76	181.64	59.35	340.79
Titratable acidity, c. c. N/10 NaOH	.164	.17	.166	.17	.164
pH	6.30	6.30	6.30	6.31	6.31
Fat clumping [a]	4.6	.6	3.4	.4	2.8
Surface tension, dynes	48.04	46.46	46.01	46.05	46.33
Protein stability, c. c. alcohol	3.48	5.6	5.08	5.6	5.04
Time to obtain 100 per cent overrun, minutes	11.77	9.30	9.66	8.8	10.82
Total score [b]	73.51	72.24	71.21	72.31	71.21

[a] 0=none, 6=most.
[b] Perfect=80. Average of scorings by 3 judges.

The mixes in which butter was used exhibited very high viscosities except in cases where the higher temperatures were employed. The higher pasteurizing temperatures considerably reduced the viscosity, as did also the combination of high pasteurizing and high homogenizing temperatures. This is particularly apparent when process 4 is compared with process 1. For some reason process 5 did not show the reduction in viscosity that appeared in process 3. Process 4 showed the least clumping, and process 2 was next. Although process 5 exhibited a greater viscosity than 3, the fat-clumping tendency was considerably less. In this instance the viscosity and fat clumping did not show the usual relationship.

The high viscosity noted in process 1 had its effect on the freezing of the mixes. The mixes of high viscosity froze more slowly than those of lower viscosity.

This experiment shows conclusively that pasteurizing and homogenizing temperatures of 170° and 180° F. save considerable time in freezing. The difference between processes 1 and 4 was nearly three minutes. For some reason process 5, which had the advantage of a high pasteurizing temperature, did not produce as good freezing results as process 3, in which the mix was heated at a lower temperature but homogenized at the same temperature (150°).

The stability of the protein in these trials varied with the temperatures used. The protein was considerably more stable in the mixes heated to 170° and 180° F., particularly when the mixes were homogenized at these temperatures. Since Doan (*3*) has shown that there is an inverse relationship between fat clumping and protein stability, this phenomenon was expected, although it was not pronounced in the experiment recorded in Table 2.

EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 10.5 PER CENT FAT MIXES WHEN THE VAT METHOD OF HEATING WAS USED

It has been clearly demonstrated that the higher temperatures of pasteurization and particularly of homogenization reduce fat clumping considerably. Since 10.5 per cent fat mixes do not form fat clumps as readily as 12 per cent fat mixes, it was thought that possibly the high temperatures would not affect these mixes to the same extent. Eight series of mixes testing 10.5 per cent fat and containing the same total solids content as the 12 per cent mixes were studied. One-half of these mixes contained butter and the other half contained cream.

Fat clumping was considerably less prominent in these mixes than in the 12 per cent mixes, which no doubt had much to do with the lower viscosity obtained. While clumping was plainly evident in these mixes, it was more pronounced in the mixes heated at the lower temperatures. The high temperatures caused a pronounced reduction of clumps, but again the most notable reduction was obtained in the mixes which were pasteurized and also homogenized at the higher temperatures. A temperature of 180° F. produced the greatest reduction of fat clumps.

It will be observed in Table 4 that the viscosity of these mixes was generally lower than that of the 12 per cent mixes, and that the higher temperatures to which the mixes were subjected reduced the viscosity. The importance of homogenizing at the higher temperatures may be noted again.

TABLE 4.—*Effect of processing temperatures on physical, chemical, and freezing properties of 10.5 per cent fat mixes, when using the vat method of heating*

[Average of eight trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, °F.	150	170	170	180	180
Homogenizing temperature, °F.	150	170	150	180	150
Viscosity, centipoises	143.06	77.72	106.61	72.86	107.24
Titratable acidity, c. c. N/10 NaOH	.196	.181	.186	.186	.181
pH	6.31	6.30	6.30	6.31	6.31
Fat clumping [a]	2.37	.87	2.12	.625	1.88
Surface tension, dynes	46.85	46.46	46.99	46.73	46.87
Protein stability, c. c. alcohol	6.0	6.1	5.85	5.8	5.95
Time to obtain 100 per cent overrun, minutes	8.9	8.5	8.61	8.16	8.7
Total score [b]	73.11	73.02	73.04	73.05	73.11

[a] 0=none, 6=most.
[b] Perfect=80. Average of scorings by 3 judges.

No striking difference was found in the protein stability as a result of the high temperatures. A great difference was not expected, if fat clumping plays an important rôle in protein stability, as the fat clumping in these mixes was not particularly prominent.

While the highest temperatures, 170° and 180° F., produced the overrun in the shortest time, the difference between the longest and shortest time was only about three-fourths of a minute. The difference in the 12 per cent fat mixes was more noticeable.

The data presented in Table 4 indicate that the beneficial effects of high pasteurizing and homogenizing temperatures are not so pronounced in the mixes of lower fat content as in these of higher fat content. If the time saved in freezing the mix is always as slight as

it was in this case, it would not be advisable to pasteurize a mix of this composition above 150° F. because of the cost of additional heat and cooling units.

EFFECT OF THE PROCESSING TEMPERATURES ON SOME PROPERTIES OF 10.5 PER CENT FAT MIXES WHEN THE CONTINUOUS METHOD OF HEATING WAS USED

Five series of 10.5 per cent fat mixes were represented in this experiment. Three of the series contained butter and two contained cream as the source of fat. One mix contained dry skim milk as a source of extra serum solids, while the remaining mixes contained condensed skim milk. Again the highest temperatures caused reduction in viscosity, but the mixes heated to 180° F. and cooled to 150° before homogenizing were higher in viscosity than the mixes heated to 170° and cooled to 150° before homogenizing. Similar results were also obtained with the 12 per cent fat mixes pasteurized by the continuous method. (Table 5.)

TABLE 5.—*Effect of pasteurizing and homogenizing temperatures on physical, chemical, and freezing properties of 10.5 per cent fat mixes when using the continuous method of heating*

[Average of five trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, °F.	150	170	170	180	180
Homogenizing temperature, °F.	150	170	150	180	150
Viscosity, centipoises	600.89	121.26	126.19	75.85	134.48
Titratable acidity, c. c. N/10 NaOH	.17	.176	.185	.192	.188
pH	6.24	6.26	6.26	6.28	6.27
Fat clumping [a]	3.6	.2	1.6	.2	1.8
Surface tension dynes	48.49	47.02	46.54	46.19	46.61
Protein stability, c. c. alcohol	4.84	6.0	5.64	5.72	5.68
Time to obtain 100 per cent overrun, minutes	9.27	7.08	6.92	6.89	8.09
Total score [b]	72.78	70.99	71.28	71.16	70.98

[a] 0=none, 6=most.
[b] Perfect=80. Average of scorings by 3 judges.

A noticeable reduction in surface tension due to high temperatures was obtained in this series. Little difference has been noted in surface tension previously, except when the mixes were pasteurized by the continuous method.

The degree of fat clumping was practically in the same order as that noted in Table 4, except that 170° F. produced the same amount of clumping reduction as 180°. When the mixes were pasteurized at the higher temperatures and cooled back to 150° for homogenization, the mixes heated to 170° produced greater reduction of clumping than the ones heated to 180°.

The stability of the proteins followed about the same order in this series as in the series pasteurized by the vat method.

An improvement in the overrun was noted in this series as compared to the overrun in the previous 10.5 per cent series heated by the vat method. The mixes heated to 150° F. were more viscous than in the former series, and the reduction in viscosity caused by the higher temperatures was greater in the case of the mixes pasteurized by the continuous system, which may account for the improvement in overrun in this experiment.

While it has been stated before that there is no direct relationship between viscosity and overrun, it appears that the relationship is

quite definite if the viscosity is due largely to fat clumping. When a mix exhibits a high degree of clumping and viscosity, destruction or partial reduction of the clumps and viscosity by high homogenizing temperatures tends to speed up the overrun. In the experiment recorded in Table 5, the saving in freezing time due to the use of high temperatures was 25 per cent, which is considerable. The viscosity of the 150° F. mixes in Table 5, while greater than the corresponding mixes in Table 4, was also greater than some of the 12 per cent fat mixes. This mix showed that the pronounced fat clumping was the cause of the high viscosity, but why this mix should have exhibited greater clumping than mix No. 1 of Table 4 is not known. Similar results have been observed in previous studies on fat clumping.

EFFECT OF THE PROCESSING TEMPERATURES ON THE PROPERTIES OF MIXES MADE FROM BUTTER

It has been observed (*1*) that mixes containing butter exhibit a greater tendency to clump than mixes made from cream. With the increased clumping there is usually increased viscosity and also a tendency to slower freezing. Whitaker (*10*) suggested that the inferior whipping ability of mixes deriving their fat from butter may be due to the lecithin deficiency of these mixes. Lecithin obtained from the soybean has been used by one of the authors in amounts ranging from 0.1 to 0.4 per cent of the mix without any beneficial results so far as overrun is concerned.

The degree of clumping in mixes deriving their fat from butter may be reduced considerably by the use of high temperatures. The greatest reduction of clumping and the greatest saving in freezing time of mixes containing butter have been obtained in the trials in which the continuous system of heating was used. Table 6 indicates the saving in freezing time because of the high temperatures used.

TABLE 6.—*Effect of processing temperatures on properties of 12 per cent mixes containing butter as the source of fat and made by the vat method of heating*

[Average of four trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, ° F.	150	170	170	180	180
Homogenizing temperature, ° F.	150	170	150	180	150
Viscosity, centipoises	373.08	133.30	262.85	82.25	238.4
Titratable acidity, c. c. N/10 NaOH	.18	.17	.177	.167	.165
pH	6.31	6.30	6.50	6.33	6.31
Fat clumping [a]	4.5	1.2	2.5	.5	2.2
Surface tension, dynes	45.95	46.39	46.50	46.23	46.03
Protein stability, c. c. alcohol	4.4	4.9	4.8	5.1	4.8
Time to obtain 100 per cent overrun, minutes	8.69	7.74	8.28	7.3	8.12
Total score [b]	70.74	70.54	70.60	70.62	70.66

[a] 0=none, 6=most.
[b] Perfect=80. Average of scorings by 3 judges.

It will be observed that the temperature of 170° and 180° F. when used for both homogenizing and pasteurizing reduced the degree of clumping and the viscosity and speeded up the freezing operation. The results listed in Table 6 were obtained from mixes pasteurized by the vat system of heating, and indicate that when the butter mixes are processed at higher temperatures a substantial saving in time is effected. In another series of trials in which butter mixes were processed by the continuous method of heating, they froze considerably

more slowly. The gain in time due to processing at the higher temperature was considerable, but because of the extremely slow freezing time of the butter mixes processed at 150° the gain was not sufficient to permit the mixes to be frozen as rapidly as previous mixes. Nevertheless, the high temperatures reduced the freezing time four and one-half minutes, as may be seen in Table 7.

TABLE 7.—*Effect of processing temperatures on properties of 12 per cent mixes containing butter as the source of fat and made by the continuous method of heating*

[Average of three trials]

Process No.	1	2	3	4	5
Pasteurizing temperature, °F.	150	170	170	180	180
Homogenizing temperature, °F.	150	170	150	180	150
Viscosity, centipoises	3,667.5	87.83	156.3	50.76	383.2
Titratable acidity, c. c. N/10 NaOH	.147	.16	.155	.16	.155
pH	6.28	6.19	6.29	6.31	6.29
Fat clumping [a]	5.3	.3	2.3	0	2.3
Surface tension, dynes	44.71	46.94	46.66	46.54	46.81
Protein stability, c. c. alcohol	3.06	5.7	5.06	5.6	5.06
Time to obtain 100 per cent overrun, minutes	14.33	10.77	11.33	10.03	13.16
Total score [b]	70.74	71.3	69.58	71.47	69.97

[a] 0=none, 6=most. [b] Perfect=80. Average of scoring by 3 judges.

The tremendous viscosity in process 1 no doubt accounted for some of the delay in freezing. The fat clumping was greatest in this mix when compared to the other mixes containing butter, but was no greater than has been noted in some other mixes. All of the butter mixes were very slow to freeze. Neither clumping nor viscosity could account for this in all cases, as the mixes processed at the high temperatures were practically without clumps and the viscosity was quite low. The acidity, freezing conditions, and all other factors that might have affected overrun were apparent satisfactory. Similar cases are often found where mixes for some unaccountable reason are slow to freeze and whip.

The main object of the experiment was to determine the effect of processing temperatures, and the results obtained demonstrate that freezing time is saved by using high processing temperatures, particularly for homogenizing.

TABLE 8.—*Effect of the processing temperature on time required to obtain a 100 per cent overrun in 12 and 10.5 per cent mixes made from cream and butter*

		12 per cent mixes					10.5 per cent mixes				
Pasteurizing temperature, °F.		150	170	170	180	180	150	170	170	180	180
Homogenizing temperature, °F.		150	170	150	180	150	150	170	150	180	150
Method of heating	Source of fat	Minutes required to obtain 100 per cent overrun									
Continuous	Cream	7.44	7.09	7.15	6.95	7.31	7.33	6.75	7.25	7.88	7.43
	Butter	14.33	10.77	11.33	10.03	13.16	10.50	7.30	6.70	6.56	8.50
Vat	Cream	7.83	7.06	7.43	7.16	7.11	8.54	8.46	8.02	7.46	7.78
	Butter	8.69	7.74	8.28	7.30	8.12	9.21	8.55	9.23	8.87	9.63
Average of cream mixes		7.63	7.07	7.29	7.05	7.21	7.93	7.60	7.63	7.67	7.60
Average of butter mixes		11.51	9.25	9.80	8.66	10.18	9.85	7.96	7.96	7.91	9.06

A comparison of the time required to obtain 100 per cent overrun of cream and butter mixes by the two methods of heating is shown in Table 8. An average of all of the 12 per cent cream and butter mixes frozen is also listed. It will be seen that the mixes containing butter yielded the desired overrun more slowly than those containing cream. The high processing temperatures caused a greater reduction of freezing time in the mixes containing butter as a source of fat than in the mixes containing cream. No doubt the reduction of viscosity and clumping was responsible for the saving in freezing time.

Processing the 10.5 per cent mixes containing butter at high temperatures produced results similar to those produced in the 12 per cent mixes. The viscosity of the 10.5 per cent mixes was lower than that of the 12 per cent mixes in most cases, however.

When the overrun of the 10.5 per cent butter mixes is considered, it is noted that high processing temperatures reduce the time needed to obtain the desired overrun in mixes made from butter. A saving of nearly four minutes may be observed in Table 8. This saving occurred in the series of mixes pasteurized by the continuous method; by the vat method the saving in time was approximately one-half minute. At the higher temperatures of processing the difference in freezing time between mixes made from cream and those made from butter was slight.

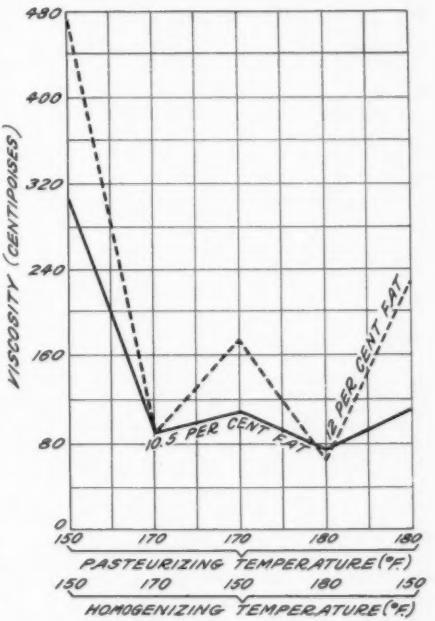

FIGURE 1.—Effect of processing temperatures upon the viscosity of 10.5 and 12 per cent fat mixes

COMPARISON OF THE EFFECTS OF HIGH PASTEURIZING AND HIGH HOMOGENIZING TEMPERATURES

As stated earlier, one of the objects of this study was to determine whether the beneficial results of high processing temperatures were due to the pasteurizing or homogenizing temperatures or to a combination of the two. From the data presented in the previous tables it is plain that high processing temperatures were beneficial, the greatest benefits being obtained when the mixes were passed through the homogenizer at the highest temperatures. It does not necessarily follow that the homogenizing temperatures were entirely responsible for these results. The individual effects of the two processes may be plainly seen in Figures 1 to 4, where viscosity, fat

clumping, speed of freezing, and protein stability are considered. These figures represent an average of all 10.5 and 12 per cent mixes treated by both the vat and continuous systems, and include over 60 mixes for each composition.

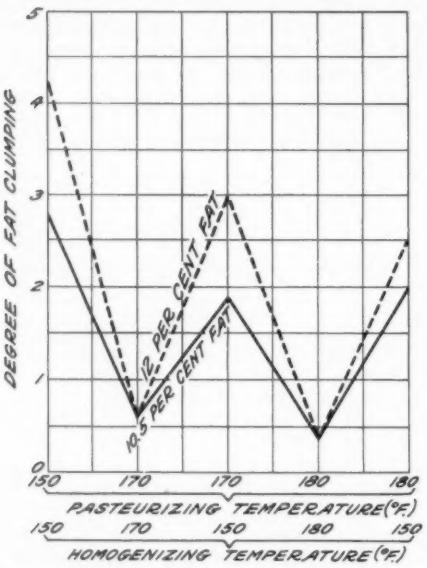

FIGURE 2.—Effect of processing temperatures upon the degree of fat clumping in 10.5 and 12 per cent fat mixes

Figure 1 shows that the temperature of heating accounted for the greatest reduction in viscosity. The reduction in viscosity due to high pasteurizing and homogenizing temperatures combined was approximately 410 centipoises in the 12 per cent mix; the pasteurization temperature of 180° F. accounted for a maximum reduction of 305 centipoises, while the homogenizing temperature accounted for a reduction of 105. In the 10.5 per cent mixes the combination of high pasteurizing and homogenizing temperatures brought about a reduction of 240 centipoises, of which the high pasteurization temperature accounted for 198 and the high homogenization temperature for 42.

The temperature of homogenizing was responsible for a greater reduction in fat clumping than the temperature of pasteurization. (Fig. 2.) This seems inconsistent with our knowledge of fat clumping and viscosity. A great deal of the viscosity of mixes is due to fat clumping, and since homogenization temperatures reduced clumping more than did pasteurizing temperatures, it would be expected that homogenization temperatures would affect viscosity more than pasteurization temperatures, but such was not the case, as may be noted. The combination of pasteurizing and homogenizing temperatures reduced the clumping from 4.2 to 0.4, or a total of 3.8 in the 12 per cent mixes. High homogenizing temperatures accounted for a reduction of 2.6, while high pasteurizing temperatures were responsible for a reduction of 1.2. In the 10.5 per cent mixes the reduc-

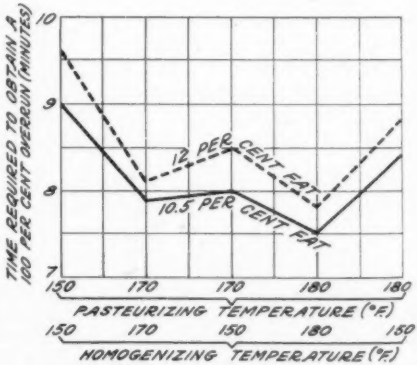

FIGURE 3.—Effect of processing temperatures upon the overrun of 10.5 and 12 per cent fat mixes

tion due to the combination of high pasteurizing and homogenizing temperatures was not as great. This was expected because of the lower fat and higher serum solid content of the mix. The combined reduction was from 2.8 to 0.4, or a total of 2.4, 1.5 being due to the homogenizing temperature and 0.9 to the pasteurizing temperature.

In Figure 3 it will be noted that the reduction in freezing time due to the combined effects of pasteurizing and homogenizing temperature was 1.8 minutes in the 12 per cent mixes, a maximum of 1.1 minutes being due to the pasteurizing temperature and 0.7 of a minute to the homogenizing temperature. A total reduction of 1.4 minutes was obtained in the 10.5 per cent mixes; a reduction of 1.1 minutes being due to the pasteurizing temperature and only 0.3 of a minute to the homogenizing temperature.

Figure 4 shows that protein stability was influenced by the higher temperatures used. The range of difference indicates that the pasteurization temperature was more responsible for the change than the homogenizing temperature. In the 12 per cent mixes the total difference was 1.05 c. c. of alcohol, 0.8 c. c. being due to the pasteurizing temperature and only 0.25 c. c. to the homogenizing temperature. In the 10.5 per cent mixes a smaller difference was noted, as would be expected, since there appears to be an inverse relationship between the degree of fat clumping and protein stability. It will be remembered that the protein stability difference due to high temperatures in the 10.5 per cent mixes was less than in the 12 per cent mixes.

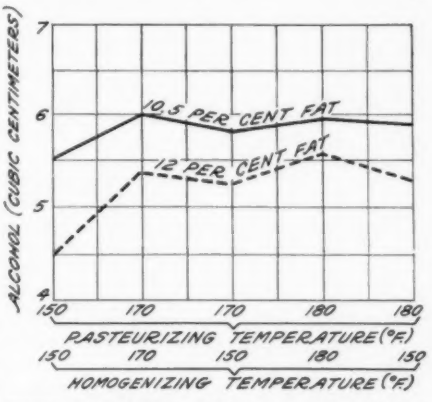

FIGURE 4.—Effect of processing temperatures upon the protein stability of 10.5 and 12 per cent fat mixes

When Figures 1 to 4, inclusive, are examined it will be observed that the mixes pasteurized at 180° F. and homogenized at 150° did not show the beneficial effects that were obtained in the mixes pasteurized at 170° and homogenized at 150° when viscosity and freezing time are considered. Yet the mixes pasteurized and homogenized at 180° benefited more than mixes processed entirely at 170°. Most of this difference occurred in the mixes heated by the continuous system.

SUMMARY

The object of the study reported in this paper was to determine the effects of high processing temperatures on certain properties of the ice cream mix, and to study the feasibility of using the flash or continuous system as means of obtaining the high temperatures.

Processing the mix at temperatures of 170° and 180° F. decreases the degree of fat clumping, reduces viscosity and freezing time, and increases protein stability.

The differences obtained between temperatures of 170° and 180° F. is not sufficiently great to warrant using processing temperatures much in excess of 170°.

The greatest benefits to be derived from the use of these high temperatures result when the mix is both pasteurized and homogenized at the high temperatures.

Lowering the temperature of the mix from 170° and 180° F. to 150° F. for homogenizing neutralizes some of the benefits obtained, particularly when the mixes heated to 180° are reduced to 150°.

The continuous system of heating is a simple method for obtaining high temperatures. All of the ingredients may be mixed and heated to 145° or 150° F. in a vat. The temperature can then be raised quickly to 170° or 180° F. by means of a continuous heater just before the mix is passed through the homogenizer.

The high pasteurizing temperature has a greater effect on viscosity, overrun, and protein stability than the high homogenizing temperature, whereas the high homogenizing temperature has a greater effect on fat clumping than the high pasteurizing temperature.

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A STUDY OF THE COTTON PLANT WITH ESPECIAL REFERENCE TO ITS NITROGEN CONTENT [1]

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INTRODUCTION

Although cotton has been extensively studied, comparatively little was known regarding its physiology, especially its nitrogen relations, until recently (*4, 5, 12, 13, 14, 15, 16*).[2] The effect of various conditions of nitrogen fertility upon plants closely spaced—the culture now rather generally practiced under boll-weevil conditions—as compared with those widely spaced, and the stage of growth at which the greatest absorption of nitrogen takes place are important agronomic problems in connection with fertilizer applications and cultural practices. The dry-weight relations of the vegetative parts of the plant, such as leaves, stalks, and roots, as compared with fruits, as well as their nitrogen content, are important in influencing the summation of plant activities expressed in crop yield. This paper presents the results of a study of these factors. The findings here reported were obtained while the writers were engaged in a study of the fruiting habits of the cotton plant, and represent one phase of the investigations carried on at the Pee Dee Experiment Station, Florence, S. C., during the past few years. Data obtained in 1925 to 1927, inclusive, bearing on the dry weight and total nitrogen relations of the various plant parts are presented, as well as the data on the dry weight and total nitrogen of the developing fruit secured in 1928.

WORK OF OTHER INVESTIGATORS

The chemical analyses of cotton plants made by different workers are not easily compared since there are variations in the percentages ot the constituents, evidently due to different varieties, soils, fertilizer practices, and climatic conditions. McBryde and Beal (*10*), White (*20, 21*), Blagoveschenski et al. (*4, 5*), and Ross (*18*) have found that the percentage of nitrogen varies with the stage of plant development. The analyses by Anderson (*1*), Fraps (*6*), McBryde (*9*), McHargue (*11*), and Williams (*22*), made only at one stage of growth, fail to reveal the gradual changes in composition during development although they show differences in the percentage composition of certain elements and compounds as well as appreciable differences in the proportions of stalks, roots, and leaves as compared with seed and lint. The analyses at different stages of growth reported by the above workers undoubtedly account for some, though probably not all, of the variations observed.

Appleton and Helms (*2*) have studied the rate of absorption of nitrogen by cotton from applications of nitrate of soda and their findings are mentioned in connection with the discussion of data.

[1] Received for publication July 28, 1930; issued May, 1931. Technical contribution No. 2 (new series) from the South Carolina Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 702.

Pate (*17*) made a preliminary report on the rate of absorption of nitrate of soda by cotton.

Maskell and Mason (*12, 13, 14, 15, 16*) have recently published extensive studies on the transport of various forms of nitrogen in the tissues of the cotton plant.

EXPERIMENTAL METHODS

All the plants used in this work were grown from seed of one strain of the Cleveland variety which had been carefully tested and selected for uniformity.

Unless otherwise stated, the samples used in 1925, 1926, and 1927 consisted of eight plants each. The large size attained by the plants toward the end of the season made it almost a physical impossibility, under the conditions that existed, to use more than eight plants in a sample, although a greater number would have been desirable. Two large electric drying ovens and three smaller ones were at times taxed to capacity because of the quantity of material. The aid of six to eight assistants was necessary to clean, separate, and weigh the plant parts in a relatively short time so that all material might be placed in the ovens before an appreciable loss of moisture or considerable enzymatic changes could occur.

Samples were obtained at weekly intervals from the middle of June until the latter part of August in 1925 and 1926. Sampling began with the appearance of flower buds or "squares" and ceased when the plants retained no more young bolls. Four plants carefully selected for uniformity were taken from each of two areas in the plot. In 1927, one plant as it occurred in the row was taken from eight previously selected areas. Thus the personal element in the selection was somewhat lessened. The plants were pulled from the ground about 9 a. m., wrapped in moist cheesecloth and quickly brought to the laboratory, where sand and other foreign particles were brushed from them. Special care was taken to give all contrasted samples identical treatment. Except for a limited time in 1926, when roots also were gathered, only the aerial portions of the plants were used, these being separated into leaves, stalks, and fruits. Both green and dry weights were obtained. After the samples had been dried at 65°–70° C. to avoid carbohydrate decomposition (*8*), they were coarsely ground and stored in mason fruit jars until analyses could be made. They were then reground to pass an 80-mesh sieve, and 1-gm. or 2-gm. duplicate portions were analyzed by the Kjeldahl method (*3*) modified to include nitrate nitrogen.

In 1925 and 1926 plats were spaced 6 and 24 inches apart in different rows, but in 1927 and 1928 only a 12-inch spacing was used. In 1925, 1927, and 1928 plants were grown on a Norfolk sandy loam which was known to give the very low yield of less than one-half bale of cotton per acre unless liberal applications of nitrogen, phosphorus, and potassium were made. In 1926 the plants were grown on a soil of the same type which gave the relatively high yield of slightly less than one bale per acre.[3]

Relatively large and small amounts of nitrogen were applied to the plots in the first three years, but only the larger application was made in 1928.

[3] It was impossible to obtain exact yields for these plots since some of the plants were used for chemical analyses.

The plot selected in 1925 contained a very unproductive soil, and it was thought best to use 200 pounds of a 4–8–4 fertilizer per acre on the low-nitrogen plot before planting in order to obtain plants not too seriously retarded in growth. The high-nitrogen plot received 1,080 pounds of the same fertilizer per acre. Subsequent applications of nitrate of soda are indicated in Table 10.

A plot of fairly productive soil was selected in 1926, and in order to get contrasts in plant growth, no fertilizer was added to the low-nitrogen plot, while 1,380 pounds of a 4–8–4 fertilizer per acre were added to the high-nitrogen plot before planting. Subsequent applications of nitrogen are explained in Table 11.

In 1927 the same amounts of phosphorus and potash were added to both plots, only the nitrogen being varied. The amounts applied are shown in Table 12.

PRESENTATION OF DATA

DRY-WEIGHT RELATIONS OF THE PLANT PARTS

The percentages of dry matter in the leaves and stalks of plants grown in 1925 and 1926 under high-nitrogen and low-nitrogen conditions and with 6-inch and 24-inch spacings are given in Tables 1 and 2. There are considerable variations between approximately the same dates in 1925 and 1926, and even from week to week in the same season. There was, however, a distinct tendency for the leaves and stalks of plants from the low-nitrogen plot to be appreciably higher in percentage of dry matter, and therefore less succulent than those of plants from the high-nitrogen plot. This is in accord with facts established for many other plants, in which increased nitrogen is associated with increased succulency. With increasing maturity, plants from both the high-nitrogen and low-nitrogen plots tended to have higher percentages of dry matter. The boll data show the same general trend as those of the leaves and stalks, and so have been omitted.

TABLE 1.—*Percentages of dry matter in cotton leaves from plants differently spaced and fertilized, 1925 and 1926*

Spacing, plot treatment,[a] and year	Percentage of dry matter in leaves sampled—																	
	June 18	June 25	June 29	July 1	July 6	July 8	July 14	July 15	July 21[b]	July 22	July 28[b]	July 29	August 4[b]	August 5	August 11[b]	August 12	August 18[b]	August 19	August 26
24 inches, HN:																			
1925			18.3		18.3		22.0		20.4		22.0		23.6		24.1		31.9		
1926	17.5	16.9		16.2		17.8		27.7		22.3		20.7		20.2		22.4		18.0	21.9
24 inches, LN:																			
1925			18.6		18.7		18.0		22.2		23.9		18.2		27.5		26.7		
1926	19.3	19.4		18.5		21.4		19.8		25.5		24.2		23.7		24.4		25.4	20.3
6 inches, HN:																			
1925			17.4		17.9		19.9		20.5		24.1		25.1		28.8				
1926	19.9	17.6		15.3		18.7		17.9		21.5		21.7		20.7		21.1		24.5	23.1
6 inches, LN:																			
1925			19.5		21.1		22.2		23.0		28.0		28.0		31.8		29.7		
1926	21.1	18.5		17.2		20.7		19.5		23.3		23.3		25.6		25.2		25.6	28.2

[a] HN=high nitrogen, LN=low nitrogen.

[b] Sample of six plants each.

TABLE 2.—*Percentages of dry matter in cotton stalks from plants differently spaced and fertilized, 1925 and 1926*

Spacing, plot treatment,[a] and year	Percentage of dry matter in stalks sampled—																	
	June 18	June 25	June 29	July 1	July 6	July 8	July 14	July 15	July 21[b]	July 22	July 28[b]	July 29	August 4[b]	August 5	August 11[b]	August 12	August 18[b]	August 19	August 26
24 inches, HN:																			
1925			14.6		16.9		17.9		20.7		23.3		28.1		31.1				
1926	16.4	14.9		12.8		17.0		18.9		24.9		26.2		24.4		25.1		28.7	29.3
24 inches, LN:																			
1925			15.7		16.4		20.6		21.1		28.1		32.3		34.7		26.8		
1926	16.8	16.3		16.7		21.2		14.2				26.8		26.2		27.7		31.1	29.5
6 inches, HN:																			
1925			14.9		17.8		20.6		24.5		32.1		34.3		36.6		34.2		
1926	17.9	15.6		12.5		18.5		18.3		27.0		27.7		25.2		38.1		33.0	30.4
6 inches, LN:																			
1925			12.4		22.9		25.0		22.0		42.5		37.6		40.7		36.3		
1926	20.4	16.5		15.1		20.1		22.5		28.5		32.0		32.4		33.0		33.7	33.4

[a] HN = high nitrogen, LN = low nitrogen. [b] Sample of six plants each.

TABLE 3.—*Percentage of the total dry weight in each tissue of cotton plants differently spaced and fertilized in 1925*

Part of plant, spacing, and plot treatment[a]	Percentage of total dry weight of samples taken—							
	June 29	July 6	July 14	July 21	July 28	August 4	August 11	August 18
Leaves:								
24 inches, HN	75.2	65.7	66.1	55.0	52.2	42.1	34.4	32.4
24 inches, LN	70.1	69.1	55.7	56.3	45.2	29.7	29.6	27.9
6 inches, HN	68.7	60.9	54.5	47.4	36.5	30.8	25.7	----
6 inches, LN	60.7	51.0	49.7	47.2	34.1	27.2	22.6	15.2
Stalks:								
24 inches, HN	24.8	29.5	26.3	31.1	31.8	28.8	23.9	27.2
24 inches, LN	29.9	27.3	35.3	30.7	31.9	32.6	27.4	17.2
6 inches, HN	31.3	34.6	35.1	35.4	29.3	28.6	25.8	----
6 inches, LN	39.3	44.0	43.1	36.2	36.7	33.8	37.4	23.4
Squares and bolls:								
24 inches, HN	----	4.7	7.6	13.9	16.0	29.1	41.7	40.4
24 inches, LN	----	3.6	9.0	13.0	22.9	37.7	43.0	54.9
6 inches, HN	----	4.5	10.4	17.2	34.2	40.6	48.4	----
6 inches, LN	----	4.9	7.1	16.6	29.1	39.0	40.0	61.4

[a] HN = high nitrogen, LN = low nitrogen.

TABLE 4.—*Percentage of the total dry weight in each tissue of cotton plants differently spaced and fertilized in 1926*

Part of plant, spacing, and plot treatment[a]	Percentage of total dry weight of samples taken—									
	June 18	June 25	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26
Leaves:											
24 inches, HN	77.3	73.8	73.3	61.8	64.1	48.5	38.1	36.9	36.4	35.4	25.6
24 inches, LN	78.5	72.1	69.2	61.3	64.0	45.2	39.0	35.4	31.2	27.6	21.2
6 inches, HN	76.5	73.9	70.5	56.4	55.8	43.7	34.8	35.5	30.5	23.0	21.2
6 inches, LN	77.7	71.1	65.5	60.9	52.5	39.9	29.6	31.6	24.9	19.4	18.9
Stalks:											
24 inches, HN	22.6	26.2	26.7	34.8	30.2	31.4	28.8	24.3	26.7	29.9	27.0
24 inches, LN	21.5	27.9	30.8	34.5	25.9	33.7	28.2	26.7	21.7	23.5	19.9
6 inches, HN	23.5	26.1	29.5	40.3	38.3	39.8	30.2	28.6	25.3	20.2	20.4
6 inches, LN	22.3	28.9	34.5	35.1	43.2	34.4	32.4	31.6	24.0	22.1	25.8
Squares and bolls:											
24 inches, HN	----	----	----	3.4	5.7	11.2	23.6	30.1	29.3	27.5	39.7
24 inches, LN	----	----	----	4.2	10.1	12.1	24.5	28.4	40.9	42.9	53.1
6 inches, HN	----	----	----	3.3	5.9	8.4	25.7	26.5	37.4	51.0	52.6
6 inches, LN	----	----	----	4.0	4.3	16.8	28.1	27.0	44.8	51.5	49.4
Roots:											
24 inches, HN	----	----	----	----	----	8.9	9.4	8.7	7.6	7.2	7.6
24 inches, LN	----	----	----	----	----	9.0	8.4	9.5	6.1	6.0	5.8
6 inches, HN	----	----	----	----	----	8.1	9.3	9.4	6.8	5.9	5.8
6 inches, LN	----	----	----	----	----	8.9	9.9	9.9	6.3	7.0	5.9

HN = high nitrogen, LN = low nitrogen.

Tables 3 and 4 show the relative weights of each tissue for 1925 and 1926, under the different conditions of spacing and fertility. The same general trends are evident, although there were differences between the two years, due no doubt to the effect of climatic and soil factors upon the growth and development of the plants. Early in the season prior to fruiting, leaves constituted approximately three-fourths of the dry matter of the plants, whereas only one-fifth to one-third was found in this tissue near the end of the season when the plants had their maximum load of fruit. As the proportion of leaves decreased the proportion of fruit increased steadily, and about two-fifths to three-fifths of the dry matter of the plants was in the fruit near the end of August. The proportion of stalks increased for a time and then gradually declined, although there was no such marked variation in the relative amounts as in leaves and fruits. The roots[4] comprised a relatively small proportion of the weight of the plants, in no case being more than 9.9 per cent. The proportion of roots also declined as the plants matured.

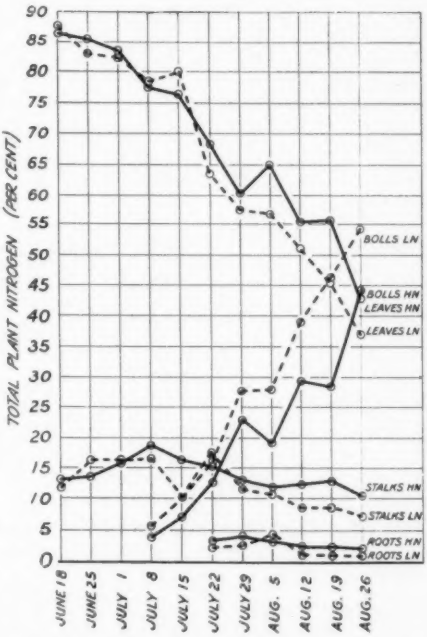

FIGURE 1.—Percentage of total plant nitrogen in leaves, stalks, and bolls of cotton plants grown in high-nitrogen (HN) and low-nitrogen (LN) plots and spaced 24 inches apart in 1926

NITROGEN RELATIONS OF THE PLANT PARTS

As previously mentioned, all plant samples were analyzed for total nitrogen. Because the results for 1925 were so similar to those for 1926 the 1925 results have been omitted and only those for 1926 and 1927 are presented. The percentage of nitrogen in each dry tissue is given in Tables 5 and 6. The amount of nitrogen per plant has also been calculated, and the percentage of the total present in each tissue is shown in Figures 1, 2, and 3.

Although there are some variations between individual samples, probably due to the use of only eight plants in a sample, the general relations as brought out in the following discussion are quite evident. The percentage of nitrogen in all plant parts was, with a few exceptions, greater under high than under low nitrogen conditions, regardless of the favorable soil and season of 1926 and the relatively unfavorable conditions of 1927.

[4] Only the larger roots were obtained.

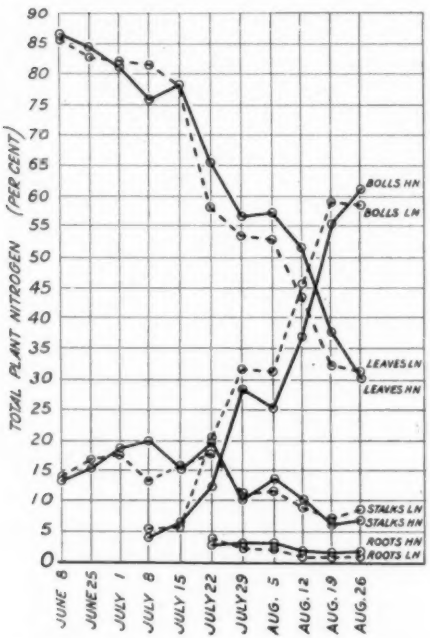

FIGURE 2.—Percentage of total plant nitrogen in leaves, stalks, and bolls of cotton plants grown in high-nitrogen (HN) and low-nitrogen (LN) plots and spaced 6 inches apart in 1926

TABLE 5.—*Percentages of nitrogen in the leaves, stalks, squares and bolls, and roots of cotton plants differently spaced and fertilized in 1926*

Part of plant, spacing, and plot treatment*	Percentage of nitrogen in samples taken—									
	June 18	June 25	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26
Leaves:											
24 inches, HN	4.69	4.90	4.75	4.68	4.74	4.25	4.00	4.25	3.97	3.68	3.62
24 inches, LN	4.57	4.24	4.02	3.61	4.34	3.53	3.11	3.80	3.26	3.14	3.12
6 inches, HN	4.43	4.66	4.88	4.26	4.71	3.76	3.76	4.32	3.97	2.99	2.78
6 inches, LN	4.40	4.22	3.84	3.46	3.62	3.82	3.52	2.78	2.52	2.35	2.12
Stalks:											
24 inches, HN	2.40	2.28	2.52	2.04	1.74	1.47	1.16	1.30	1.25	1.03	.86
24 inches, LN	2.28	2.15	1.76	1.34	1.32	.....	.88	1.01	.74	.66	.63
6 inches, HN	2.21	2.41	2.70	1.56	1.33	1.23	.84	1.34	.92	.57	.67
6 inches, LN	2.55	2.09	1.60	.98	.88	1.38	.68	.63	.57	.45	.43
Squares and bolls:											
24 inches, HN	.....	.....	.....	4.39	4.22	3.61	2.40	2.80	2.60	2.45	2.38
24 inches, LN	.....	.....	.....	3.57	3.49	3.27	2.44	2.34	1.91	2.02	1.84
6 inches, HN	.....	.....	.....	3.93	3.84	3.62	2.59	2.56	2.28	1.98	2.24
6 inches, LN	.....	.....	.....	3.48	3.35	3.19	2.22	1.93	1.46	1.63	1.56
Roots:											
24 inches, HN	.....	.....	.....	.....	.....	1.13	1.12	1.01	.82	.74	.61
24 inches, LN	.....	.....	.....	.....	.....	.81	.84	1.03	.60	.48	.43
6 inches, HN	.....	.....	.....	.....	.....	.93	.88	.95	.76	.48	.58
6 inches, LN	.....	.....	.....	.....	.....	1.02	.48	.47	.23	.39	.38

* HN = high nitrogen, LN = low nitrogen.

TABLE 6.—*Percentage of total nitrogen in the leaves, stalks, and bolls of cotton plants differently spaced and fertilized in 1927*

Part of plant, spacing, and plot treatment [a]	Percentage of nitrogen in samples taken—										
	June 16	June 21	June 25	June 28	July 1	July 4	July 7	July 10	July 13	July 17	July 21
Leaves:											
12 inches, HN	4.55	4.16	4.35	4.11	4.23	4.05	4.14	4.09	3.84	3.59	3.33
12 inches, LN	3.31	2.65	2.48	2.55	2.09	3.14	3.32	3.25	3.64	3.07	2.94
Stalks:											
12 inches, HN	2.11	1.87	1.78	1.94	1.76	1.92	1.81	1.75	1.45	1.40	.99
12 inches, LN	1.22	1.06	1.32	1.15	.90	1.20	1.00	.96	1.13	1.06	.88
Bolls:											
12 inches HN					4.25	4.35	4.17	3.93	3.82	3.77	3.11
12 inches, LN								3.19	3.45	2.69	2.67

Part of plant, spacing, and plot treatment [a]	Percentage of nitrogen in samples taken—									
	July 24	July 27	July 30	August 2	August 5	August 8	August 11	August 15	August 20	August 27
Leaves:										
12 inches, HN	3.53	3.67	3.68	3.74	3.58	3.39	3.66	2.95	3.33	3.54
12 inches, LN	3.23	3.51	3.47	2.69	3.08	2.98	3.27	3.18	3.02	3.43
Stalks:										
12 inches, HN	1.12	1.21	1.01	1.16	.88	.95	.86	.74	.67	.75
12 inches, LN	1.03	.97	1.02	.69	.96	.73	.88	.84	.72	.98
Bolls:										
12 inches HN	2.91	2.85	2.86	2.56	2.30	2.29	2.34	2.23	2.15	2.09
12 inches, LN	2.42	2.39	2.25	1.98	2.12	1.99	1.87	2.01	1.89	1.97

[a] HN = high nitrogen, LN = low nitrogen.

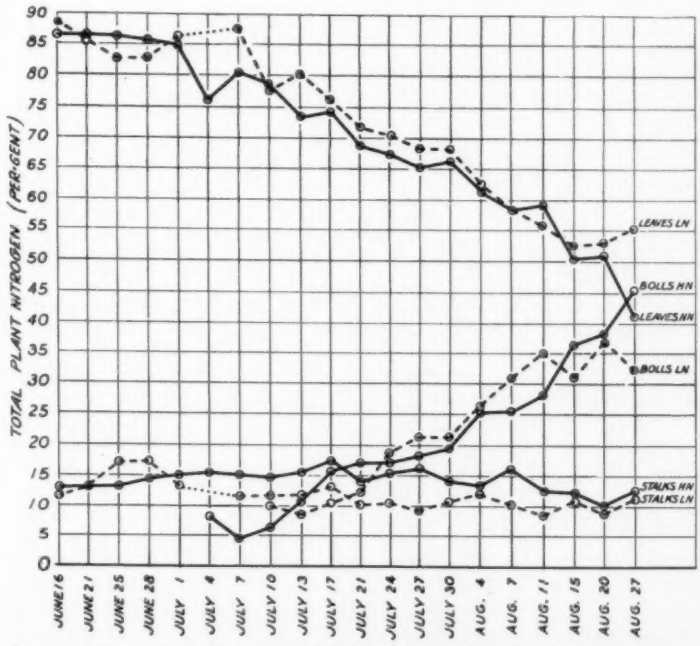

FIGURE 3.—Percentage of total plant nitrogen in leaves, stalk, and bolls of cotton plants grown in high-nitrogen (HN) and low-nitrogen (LN) plots and spaced 12 inches apart in 1927

Anderson (*1*) by applying nitrogen, phosphorus, or potash to an unproductive soil was able to increase the percentage of that constituent in cotton plants grown on it. White (*21*), on the other hand, concluded that the relative amounts of nitrogen and mineral ingredients in the plants did not appear to be greatly affected by the quantity supplied. His tables for 1911 and 1912 show, however, that when a fertilizer containing no nitrogen was applied, the percentage of nitrogen in the plants was lower than when a standard fertilizer containing nitrogen was used. Williams (*22*) reports that cotton plants grown on unfertilized soil had the highest percentage of nitrogen in the leaves and other vegetative parts. This is not in agreement with the findings of Anderson (*1*) and White (*21*), nor with those presented in this paper. It can be seen in Table 6 that one analysis of a small number of plants might lead to a conclusion such as that of Williams, since the percentages of nitrogen in leaves on August 15, and stalks on August 5, 11, 20, and 27, were smaller under high-nitrogen conditions. The conception of balance of the nutrients of the soil solution is of importance in an interpretation of such results. Thomas (*19, p. 426*) states: "However * * * experimental evidence exists to show that the concentration of nutrients of the soil solution may be the factor of greatest influence in determining the course of absorption."

It is evident from Table 5 that widely spaced plants generally show a higher percentage of nitrogen in their tissues than those closely spaced after approximately July 1, when the competitive effects of close spacing become noticeable.

The percentage of nitrogen in the tissues was generally smaller in 1927 than in 1926 even if the 12-inch spacing is compared with the more crowded conditions of the 6-inch spacing. The seasonal factors associated with a large number of cloudy days and many rains were probably of importance in this connection, since conditions in 1927 were very unfavorable for cotton production, although the soil of the plot used was less productive than that employed in 1926 and the fertilizers were also different. The unfavorable effect of the seasonal complex is strongly indicated, however, since the Cleveland 5 variety produced on an average only 1,159 pounds of seed cotton per acre on all other experiment station plots in 1927 as compared with 2,417 pounds in 1926.

An examination of Figures 1, 2, and 3 reveals the fact that the leaves contained from 80 to 90 per cent of the plant's nitrogen when the first few squares were present, and that this proportion steadily declined until 30 to 55 per cent was present the latter part of August, when approximately a full load of fruit had been produced. The load of fruit evidently affected the proportions of the total nitrogen in the leaves of plants grown on both high and low nitrogen plots in 1926 and 1927. With a large crop of fruit in 1926 a relatively smaller percentage of the total amount of nitrogen was present in the leaves of closely spaced plants grown under low-nitrogen than in those grown under high-nitrogen conditions. With a very poor retention of fruit by all plants in 1927, the leaves from plants on low-nitrogen plots had a greater proportion of the total nitrogen than did those from high-nitrogen plots, even though there was a greater evident nitrogen starvation of the plants grown under low-nitrogen conditions.

The fruits from the plants on low-nitrogen plots showed in 1926 approximately 55 per cent of the total nitrogen, whereas in 1927 they contained only 37 per cent. That the cotton fruits made rapid demands for nitrogen is shown by the fact that 40 to 60 per cent of the entire amount of this constituent was found in the fruits the latter part of August, whereas relatively minor proportions were present four or five weeks earlier. Maskell and Mason (*12*) in their first paper on the transport of nitrogenous substances in the cotton plant infer that nitrogen goes very largely to the bolls and little to the roots. This was found to be true in the studies here reported, as will be shown later. Figures 1 and 2 show that closely spaced plants had an appreciably greater proportion of the nitrogen in the fruits than had the widely spaced plants.

The roots contained the smallest amount of nitrogen, this constituent never exceeding 4.1 per cent of the total and in some instances being as low as 1.0 per cent. As may be seen in Table 5, there were only slight differences in the percentage of nitrogen in the roots of plants from high and low nitrogen plots. In all the plants the proportion of nitrogen in the roots declined appreciably as the plants matured. The stalks also contained a small proportion of the total nitrogen, in some cases as little as 7 per cent, at the time when practically no more fruit buds and bolls were being retained. The bolls and leaves accumulated by far the greatest amount of nitrogen both in total nitrogen and in percentage concentration.

As is shown in Table 5, some very distinct differences in the percentage of nitrogen in composite samples of squares and bolls of plants grown under high-nitrogen and low-nitrogen conditions were observed in the late samples of 1926. It therefore became of interest to know whether these differences also existed in the seed. Williams (*22*) had found that a high nitrogen content of the seed was associated with the use of fertilizers having a high nitrogen and phosphorus content. Samples of August 19 and August 26, therefore, were divided into boll walls (carpels), lint, and seed, and nitrogen analyses were made. The results are presented in Table 7. It should be remembered that the bolls were of varied and unknown ages, many being almost mature, and that the closely spaced plants of the low-nitrogen plots were more mature than those of the high-nitrogen plots. There were no significant differences in the percentage composition of nitrogen in the seed with the exception of the sample collected August 26 from the close spacing on the high-nitrogen plot. It is possible that the relatively high percentage of nitrogen in the seed of plants from this plot was due to a sampling error. The low percentage of nitrogen in the boll walls and lint of both the widely and closely spaced plants from the low-nitrogen plots is consistent and quite evident. The data of this table seem to indicate that the nitrogen content of the seed was maintained at approximately the same concentrations, even though tissues such as leaves, stalks, boll walls, and lint showed a relatively low nitrogen content with a low supply of nitrogen.

Further studies of the fruits were made in 1928 by analyzing a series of bolls 7, 10, 13, 17, and 21 days of age from plants grown under high-nitrogen and low-nitrogen conditions and spaced 12 inches apart. The data are presented in Tables 8 and 9. There were no signifi-

cant differences in the nitrogen content of entire bolls, the separate walls, or seed and lint during the first 21 days, a period in which the bolls had attained full size but not one-half their maturity. It may be inferred that the differences in composition of the boll walls and lint in 1926 (Table 7) were due to the greater maturity of those samples.

TABLE 7.—*Percentages of nitrogen in boll walls, lint, and seed of cotton plants differently spaced and fertilized, 1926*

Tissue	Date of sampling	Percentage nitrogen when spacing and plot treatment were as indicated			
		24 inches, HN[a]	24 inches, LN[a]	6 inches, HN[a]	6 inches, LN[a]
Boll wall	Aug. 19	2.35	1.75	1.73	1.04
	Aug. 26	2.49	1.40	1.84	.96
Lint	Aug. 19	1.17	.70	.73	.38
	Aug. 26	.78	.47	.58	.53
Seed	Aug. 19	3.18	3.27	2.97	2.85
	Aug. 26	3.29	3.18	3.51	2.67

[a] HN=high nitrogen, LN=low nitrogen.

TABLE 8.—*Percentages of nitrogen in bolls, boll walls, and seed and lint from cotton plants differently spaced and fertilized, 1928*

Part of plant	Percentage nitrogen at age in days and with plot treatment indicated									
	7		10		13		17		21	
	HN[a]	LN[a]	HN[a]	LN[a]	HN[a]	LN[a]	HN[a]	LN[a]	HN[a]	LN[a]
Bolls	2.50	2.32	2.15	2.10	2.07	2.05	1.95	1.81	1.83	1.80
Boll walls	2.36	2.11	2.06	2.00	2.00	1.95	1.86	1.63	1.86	1.69
Seed and lint	2.98	3.04	2.37	2.32	2.18	2.25	2.09	2.11	1.80	1.93

[a] HN=high nitrogen, LN=low nitrogen.

TABLE 9.—*Proportions of nitrogen in each tissue of bolls from cotton plants differently spaced and fertilized, 1928*

Part of plant	Percentage nitrogen at age in days and with plot treatment indicated									
	7		10		13		17		21	
	HN[a]	LN[a]	HN[a]	LN[a]	HN[a]	LN[a]	HN[a]	LN[a]	HN[a]	LN[a]
Boll walls	74.3	68.9	64.1	64.2	59.9	63.2	58.4	57.4	47.8	50.5
Seed and lint	25.7	31.1	35.9	35.8	40.1	36.8	41.6	42.6	52.2	49.5

[a] HN=high nitrogen, LN=low nitrogen.

Though the different forms of nitrogen in the various tissues are not revealed by the analyses which have been made, yet the data presented give indications that the stalks of plants from low-nitrogen plots and all roots stored relatively little nitrogen, but the leaves and boll walls probably serve as organs for the accumulation of more nitrogen than the minimum concentration required for growth. It may perhaps be assumed that the concentration of nitrogen in the stalks and roots of plants from low-nitrogen plots, in general, is little if at all above the minimum metabolic requirements of these tissues. If, for any reason, the absorption of nitrogen ceases or is retarded, it seems probable that nitrogen present in excess of the minimum requirements in all leaves, boll walls, or stalks of plants from high-nitrogen plots may be used for the further development of the growing seed, which, as has been shown for certain ages at least, tends to

have the same percentage of nitrogen in plants grown under both high-nitrogen and low-nitrogen conditions.

Attention has been called to the relatively small quantity of the total nitrogen present in the cotton stalks. The leaves, on the other hand, contained from two to three times the amount of nitrogen in the stalks. The bolls, and particularly the seed, contained a considerable proportion of the nitrogen of the cotton plant in the latter part of the season. Under conditions of close spacing the seed may contain the bulk of the nitrogen of the crop, the leaves only one-fourth or one-fifth, and the stalks approximately one-tenth.

ABSORPTION OF NITROGEN

Information on the rapidity at which the available nitrogen in the soil is removed by cotton plants is a matter of considerable economic importance and should be of value in improving fertilizer practices by possibly reducing the loss of the soluble nitrogenous constituents. Such loss would depend upon the amount of available nitrogen which is subjected to leaching or changed into other forms. The total nitrogen in the plants calculated as pounds per acre of nitrogen is shown in Tables 10, 11, and 12, and when considered in connection with the nitrate applications gives some information as to the periods of greatest absorption of nitrogen from the soil. Certain irregularities occur in these tables, as, for example, the possible loss of nitrogen early in the season. An actual loss of nitrogen may have taken place, though variations in the 8-plant samples seem the more plausible explanation.

TABLE 10.—*Pounds of nitrogen per acre in cotton plants differently spaced and fertilized, and analyzed in 1925*

[Applications of 100 pounds of $NaNO_3$ per acre were made on May 23 to all plots, and on June 19 and July 17 to HN plots]

Spacing and plot treatment [a]	Pounds of nitrogen per acre in plants sampled—							
	June 29	July 5	July 12	July 19	July 26	August 2	August 9	August 16
24 inches, HN	5.8	19.6	31.2	43.7	58.6	80.2	91.2	79.0
24 inches, LN	7.6	12.7	26.0	33.7	61.5	48.0	52.7	57.5
6 inches, HN	23.9	43.2	64.0	70.7	87.0	89.0	69.8	53.8
6 inches, LN	32.4	27.0	36.1	43.7	40.6	50.7	50.5	41.2

[a] HN=high nitrogen, LN=low nitrogen.

TABLE 11.—*Pounds of nitrogen per acre in cotton plants differently spaced and fertilized, and analyzed in 1926*

[Applications of 75 pounds of $NaNO_3$ per acre were made on June 4 to all plots and on July 1 and July 23 to HN plots]

Spacing and plot treatment [a]	Pounds of nitrogen per acre in plants sampled—										
	June 18	June 25	July 1	July 8	July 15	July 22	July 29	August 5	August 12	August 19	August 26
24 inches, HN	2.3	3.7	8.9	22.0	32.9	39.3	46.3	81.9	86.3	79.8	69.7
24 inches, LN	2.6	6.2	7.7	13.4	20.5	35.7	37.7	52.5	56.7	83.7	64.8
6 inches, HN	8.9	13.8	24.5	45.4	80.6	70.1	73.2	83.1	144.4	132.8	194.0
6 inches, LN	5.7	12.9	18.4	31.1	35.1	61.7	53.2	56.9	61.8	83.8	65.6

[a] HN=high nitrogen, LN=low nitrogen.

TABLE 12.—*Pounds of nitrogen per acre in cotton plants differently spaced and fertilized, and analyzed in 1927*

[Applications of 75 pounds per acre of $NaNO_3$ were made to the HN plots on June 7, June 30, and July 21]

Spacing and plot treatment [a]	Pounds of nitrogen per acre in plants sampled—										
	June 16	June 21	June 25	June 28	July 1	July 4	July 7	July 10	July 13	July 17	July 21
12 inches, HN	5.4	6.1	9.5	11.5	11.7	22.7	22.4	38.0	32.5	43.6	35.2
12 inches, LN	2.2	1.8	2.9	3.0	2.5	.....	4.7	5.3	9.0	5.8	7.0

Spacing and plot treatment [a]	Pounds of nitrogen per acre in plants sampled—									
	July 24	July 27	July 30	August 2	August 5	August 8	August 11	August 15	August 20	August 27
12 inches, HN	51.4	50.1	58.5	55.4	73.2	80.9	92.3	100.4	77.8	101.3
12 inches, LN	8.4	6.6	16.5	12.3	25.5	16.7	13.8	30.6	36.9	38.5

[a] HN=high nitrogen, LN=low nitrogen.

It is seen that closely spaced plants contained larger quantities of nitrogen relatively early in the season than the widely spaced plants. The ordinary agricultural practice in South Carolina is to apply only one side application of 15 to 30 pounds of readily available nitrogen at the time of thinning. In these experiments the time of thinning was May 22. It will be noted in Table 11 that the closely spaced plants did not contain 13.5 pounds of nitrogen per acre, or the equivalent of 75 pounds of nitrogen as nitrate of soda, until June 25, even though the high-nitrogen plots had received 1,380 pounds of 4–8–4 fertilizer per acre at planting on April 20, and a subsequent application of 75 pounds of nitrate of soda per acre on June 4. The total nitrogen applied to the high-nitrogen plots in 1926 was equivalent to 97 pounds of nitrogen per acre. The analyses of widely spaced plants do not account for the total at any time, though twice this amount was found in closely spaced plants on August 26.

In general the closely spaced plants absorbed more nitrogen per acre under high-nitrogen conditions than did the widely spaced plants. However, this does not appear to have been true after August 2 (Table 10) in 1925. The decreasing nitrogen was undoubtedly due to a heavy shedding of leaves which occurred fairly early on account of dry weather and the highly competitive conditions existing between the plants spaced 6 inches apart.

Appleton and Helms (*2*) grew cotton plants in pots in the greenhouse to the stage of flower-bud production and found large increases in the percentage of nitrogen in the young plants soon after applications of nitrate of soda.

White (*20*) concluded from analyses of cotton plants at four stages of growth during four seasons that there is considerable regularity in the proportionate intake of nitrogen for each stage of development of the plant. He found that 34 per cent of the nitrogen was taken from the soil in the first period from sprouting to first flower buds, 32 per cent from first flower buds to first blooms, 18 per cent from first blooms to first open bolls, and 16 per cent from first open bolls to the maturity of all bolls. The results presented in this paper for 1926 are

in striking contrast to those of White, since 40 to 50 per cent of the nitrogen present in the plants at the end of fruit setting, but before the maturity of all the fruit, was taken up in the relatively short period of approximately two weeks. An application of nitrate of soda was made on July 23 during a very dry period, but this, as well as an earlier application, was probably not available to the plants until a rain of 2.38 inches fell on July 28. This rainfall occurred during a very active period of growth and fruit production and resulted in the rapid absorption of nitrogen from July 28 to August 12, as is shown in Table 11. This furnishes an excellent example of the possibly beneficial effects to cotton of late applications of nitrogen if the plant growth and seasonal conditions seem to demand it. Plant analyses in connection with a study of the effects of the time of application of nitrogen on final yield might partly explain some of the variable results obtained by Hall and Armstrong (7). The same amount of nitrate of soda as a side application produced the greatest yield in some years when all of it was added at the time of thinning, while in other years divided applications at thinning and the appearance of first squares gave the largest yields.

SUMMARY

Cotton plants were grown in field plots in 1925, 1926, and 1927 with a relatively low supply of nitrogen and with a relatively high supply of nitrogen.

The concentration of nitrogen in the leaves, stalks, and fruits was generally greatest where nitrogen was relatively abundant (high-nitrogen plots), and was correlated with a greater succulence of the tissues; or, conversely stated, a greater percentage of dry matter was present in the tissues of plants from plots where the nitrogen was relatively scarce (low-nitrogen plots).

The developing seed and lint from 7 to 21 days of age showed approximately the same nitrogen concentration under high-nitrogen and low-nitrogen conditions. The boll walls and lint at later stages in 1926 showed lower concentrations of nitrogen with the low nitrogen supply.

The relative proportions of the total nitrogen present in the stalks were lower in plants from low-nitrogen plots than in plants from high-nitrogen plots.

The percentage of nitrogen in all tissues except possibly seed tended to decrease with maturity.

In 1925 and 1926 closely spaced plants had a larger proportion of stalks to total dry weight and a larger proportion of their nitrogen in bolls than did widely spaced plants. Moreover, they absorbed a larger proportion of the total nitrogen relatively early and in 1926 took up considerably more nitrogen.

Just prior to the rapid production of flower buds, about three-fourths of the dry weight of the plant and from 80 to 90 per cent of the nitrogen were present in the leaves. The proportion of leaf weight to plant weight steadily declined and the proportion of boll weight to plant weight steadily increased until the end of the fruit-setting season, when only one-fifth to one-third of the dry weight of the plant and from 30 to 55 per cent of its nitrogen was present in the leaves. At this time from 40 to 60 per cent of the nitrogen of the plants was found in the bolls. At 21 days of age when bolls had

reached full size but not maturity, approximately one-half of the boll nitrogen was present in the wall tissue.

Early in the season the stalks showed an increasing proportion of the total weight and later a gradual decline, with a decrease in the proportion of nitrogen, which was as low as 7 per cent of the total on August 26, 1926.

The proportion of the root weight to the total weight was relatively small, ranging from 9.9 to 5.8 per cent during the period of root collections in 1926. The proportion of the total nitrogen was even less, ranging from 4.1 to only 1 per cent.

The total quantity of nitrogen absorbed by the plants early in the season was not large, although the rate of absorption was very rapid owing to the increase in size of the small plants. Under certain seasonal conditions, however, such as prevailed in 1926, older plants absorbed from 40 to 50 per cent of the nitrogen of the crop, equivalent to several hundred pounds per acre of nitrogen as nitrate of soda, in about two weeks.

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